

Pulmonary Platelet Trapping:

An experimental study on the influence of
endogenous opiates and prostanoids in
dogs and rabbits



Per Almqvist

Diss. Rund.
1984



From the Departments of Surgery, Malmö General Hospital,
University of Lund, Sweden and Strong Memorial Hospital,
University of Rochester, New York, USA.

PULMONARY PLATELET TRAPPING:

An experimental study on the influence of endogenous opiates and
prostanoids in dogs and rabbit.

By

Per Almqvist



Malmö 1984

This theses is based on the following papers.

- I Effect of naloxone on endotoxin-induced platelet sequestration.
Per Almqvist, Marian Kuenzig and Seymour I Schwartz.
Acta Chir Scand 149:26-26, 1983.
- II The effect of naloxone and cyproheptadine on pulmonary platelet trapping, hypotension, and platelet aggregability in traumatized dogs.
Per Almqvist, Marian Kuenzig and Seymour I Schwartz.
J Trauma 23:405-407, 1983.
- III Treatment of experimental endotoxin shock with ibuprofen, a cyclooxygenase inhibitor.
Per Almqvist, Marian Kuenzig and Seymour I Schwartz.
Circ Shock, (accepted for publication).
- IV Pulmonary platelet trapping induced by B-endorphin injection into the cerebrospinal fluid in dogs.
Per Almqvist, Karl M Knigge, Marian Kuenzig, Shirley A Joseph, Webster Pilcher and Seymour I Schwartz.
Submitted to Surgery.
- V B-endorphin administered into the lateral ventricle of the brain causes pulmonary platelet trapping in rabbits.
Per M Almqvist, Carin Larsson-Backström, Lisbet Nilsson and Ulf Haglund.
Reg Peptides, (accepted for publication).
- VI Increased survival of endotoxin shocked dogs treated with methylprednisolone, naloxone and ibuprofen.
Per M Almqvist, Björn Ekström, Marian Kuenzig, Ulf Haglund and Seymour I Schwartz.
Circ Shock, (accepted for publication).

These papers will be referred to in the text by their Roman numerals.

CONTENTS

	<u>Page</u>
Introduction.....	4
Specific aims of the study.....	4
Material and methods.....	5
Experimental design.....	8
Comments on material and methods:.....	10
A Influence of prostanoids and endorphins.....	13
Comments.....	14
B Influence of centrally administered B-endorphin.....	16
Comments.....	17
C Influence of prostanoid and endorphin blockers on survival in endotoxin shock.....	19
Comments.....	20
General discussion.....	22
Clinical implications.....	26
Conclusions.....	27
Acknowledgements.....	29
Reference list.....	30

INTRODUCTION

Non cardiac pulmonary edema, commonly called the adult respiratory distress syndrome (ARDS), is a common and serious complication of trauma, burns and sepsis, and is associated with high mortality (95, 110, 119). The etiology is complex and under considerable debate. Trapping of platelets and leucocytes in the pulmonary vasculature, release of vasoactive substances from these cells and from the endothelium, and activation of the complement, coagulation, and fibrinolytic systems seem to be of major importance (95). The relative importance of these factors is far from clear.

Pulmonary trapping of platelets in dogs subjected to trauma was demonstrated by Bergentz et al (5), and it was later shown that platelets were also trapped in the lung in endotoxic and hemorrhagic shock (31, 74). Accumulation of platelets in the lung has also been observed in humans with clinical diagnosis of ARDS (104).

The opiocortin system has, in recent years, been shown to be activated by different forms of stress, and increased amounts of circulating B-endorphin have been shown in hemorrhagic, endotoxic and traumatic shock (36, 66, 125). The endorphins have been proposed as contributors to the deleterious effects of shock, since blocking them with naloxone, an opiate antagonist, was beneficial in both hemorrhagic and endotoxic shock (26).

This research was directed to the study of the lung injury which occurs in experimental endotoxin shock and in soft tissue trauma in dogs, particularly to the contribution of pulmonary platelet trapping in this injury and the hypotension that accompanies it. The ability of B-endorphin to produce these effects and the ability of different drugs to ameliorate them, form the basis of this study.

SPECIFIC AIMS OF THE STUDY

- 1 to investigate the possible role of endorphins in the pulmonary platelet trapping (PPT) and blood pressure drop seen in dogs subjected to endotoxin shock and to soft tissue trauma, by treating the animals with naloxone, an opiate antagonist.
- 2 to investigate whether central administration of B-endorphin causes PPT and hypotension, and the possible role of humoral factors as mediators.

- 3 to investigate the role of prostaglandins derived via the cyclooxygenase pathway, on PPT and on hypotension in endotoxin shock, by treating endotoxin shocked dogs with ibuprofen, a cyclooxygenase inhibitor.
- 4 to investigate the effect of a combination of methylprednisolone, ibuprofen and naloxone; all of which have proven helpful in the treatment of experimental ARDS, on survival rate in an LD₁₀₀ canine endotoxin shock model.

MATERIAL AND METHODS

The methods used are described in the individual papers (I - VI). Additional information and a short survey is given here.

Experimental animals

A total of 126 mongrel dogs, weighing between 9 and 14 kg and 28 rabbits, weighing between 2.5 and 6 kg, were used. Both dogs and rabbits were approximately equally divided between both sexes. None of the animals had obvious signs of pulmonary or intestinal diseases. The dogs were deprived of food 12 hours before anesthesia but had free access to water.

Anesthesia

All animals were anesthetized with pentobarbital sodium. The dogs received an initial i.v. dose of 30 mg/kg b.w. and the rabbits 15 mg/kg b.w. To maintain adequate level of anesthesia the dogs were given repeated doses of 30 - 60 mg and the rabbits 15 - 30 mg as required.

Platelet tagging procedure

The dogs were anesthetized, intubated, connected to a respirator and maintained with intravenous normal saline 10 - 12 ml/kg/hr. 100 ml whole blood was removed from the saphenous vein into a Fenwal bag (Fenwal Transfer Pack, Fenwal Laboratories, Deerfield, Illinois) containing 30 ml ACD-solution and centrifuged at 250 g for 15 minutes. The platelet rich plasma was transferred to another Fenwal bag and centrifuged at 1 100 g for 20 minutes. The platelet

poor plasma was transferred to another bag, reserving 10 ml in a syringe. All syringes, stopcocks and connectors used in the tagging procedure were of plastic material to prevent loss of platelets. 10 ml of labelling solution (Ringers solution 7 ml, citrate 60 mg, glucose 50 mg, and 3 ml sterile water) was added to the platelet button and the platelets were resuspended. 400 μCi $\text{Na}_2^{51}\text{CrO}_4$ was added to the resuspended platelets and the suspension was mixed gently by inversion at room temperature for 45 minutes. The platelet poor plasma from the bag was added to the tagged platelets and mixed by inversion several times, followed by centrifugation at 1100 g for 20 minutes to remove excess ^{51}Cr . The supernatant was discarded and the platelet button resuspended in 1/3 of the reserved platelet poor plasma. Two ml of 5 % ascorbic acid was added and the suspension transferred to a plastic centrifuge tube. The bag was rinsed with two portions of the remaining platelet poor plasma and the rinse was added to the centrifuge tube. This platelet suspension was centrifuged for seven minutes at 800 g to remove erythrocytes. The supernatant containing the tagged platelets was injected into the same dog. The dogs were disconnected from the respirator, extubated and returned to the recovery room.

The amount of ACD, ^{51}Cr and ascorbic acid were increased over that suggested by Abrahamsen (2) to improve the efficiency of the tag. The final centrifugation was added to eliminate any remaining tagged red cells. Tagging was always done 24 hours prior to the shock study to allow time for damaged platelets to be removed from the circulation.

Pulmonary platelet trapping and wet/dry lung weight

Dogs: At sacrifice a two ml blood sample was taken. The lungs were excised, weighed and dried with pressurized air for five to seven days and reweighed. The pleura was peeled from the dried lungs to avoid counting surface blood. The lung parenchyma was divided and placed into tubes. ^{51}Cr activity was counted in a gamma scintillation counter (Searle Analytic Inc., Model 1195). The average of two ten minutes counts was taken for each sample and the background activity was subtracted. The ratio of counts per gram lung tissue to counts per gram blood was determined and used as a measure of pulmonary platelet trapping.

The wet/dry lung weight ratio was calculated and used as a measure of pulmonary edema.

Rabbits: The same method of determining PPT was used as in the dogs except that the lungs were not dried and the pleura was not peeled off.

Ex vivo platelet aggregability.

Dogs: Platelet rich plasma was isolated by centrifugation from citrate blood (one ml citrate to nine ml blood). ADP in concentrations 10, 50, and 100 ug/ml was added, to test the platelets' aggregability by a photoelectric method (Colysagraph Damon, Division IEC, Needham Heights, MA). The slope of the aggregation curve at base line and at the end of the experiment was compared.

Rabbits: Platelet count in the platelet rich plasma was adjusted to about 3×10^5 platelets/mm³. ADP and collagen in concentrations 10^{-4} M and 20 ug/ml respectively was added to the platelet rich plasma. The maximal slope in mm/min was calculated as a measure of inhibition or stimulation of platelet aggregability.

Drugs

- Naloxone HCL, Endo Laboratories, Garden City, New York.
- Sodium ibuprofen, 50 mg/ml, Upjohn, Crawley, Sussex, England.
- Methyl prednisolone sodium succinate (Solu-Medrol^R), Upjohn Company, Kalamazoo, Michigan.
- Cyproheptadine hydrochloride, 2 mg/ml, prepared by the pharmacy at Strong Memorial Hospital, Rochester, New York.
- Furosemide, 10 mg/ml (Lasix^R), Hoechst-Roussel Inc., Sommerville, New Jersey.

All drugs were given intravenously. The dosage is given in the description of the individual experiments.



Experimental models

Endotoxin model: Endotoxin shock was induced by an intravenous injection of either 20 mg or 2 mg/kg b.w. E. Coli endotoxin (lipopolysaccharide W.E. coli 0128:B12, control number 703722, Difco, Detroit, Michigan) in 10 ml saline over a five minute period.

Trauma model: A standard soft tissue trauma model, modified after Blalock's description was used (8). Two hundred blows with a padded mallet were administered to each hind limb.

B-endorphin model: Human B-endorphin, lipotropin 61 - 91 (Penninsula Lab, San Carlos, California) 50 ug/kg b.w. in 0.5 ml NaCl was injected into the lateral ventricle or the cisterna magna in dogs and 50 ug/kg b.w. (human B-endorphin, lipotropin 61 - 91, BaChem, Switzerland) in 0.2 ml NaCl was injected into the lateral ventricle in the rabbits.

Statistics:

The statistical analysis was done with the student's t-test in papers I and II. Wilcoxon's rank sum test for differences between groups was used in papers III - VI and the Mann-Whitney one tailed U-test was used in paper V. A p-value of less than 0.05 was considered significant. All values are reported as mean $\pm$ standard deviation.

EXPERIMENTAL DESIGN

Sham controls (given saline only) and drug control (given the drug only, in the same doses and time intervals as in the treated groups) were done for the different experimental models. The same sham control animals were used for the endotoxin and the trauma models. The same operative procedures and sampling techniques were used as in the respective models.

The endotoxin model was treated with naloxone, an opiate inhibitor, 2 mg/kg b.w. either 10 minutes before or 10 minutes after induction of endotoxin shock followed by 1 mg/kg b.w. at 30, 60 and 90 minutes postshock. A third group was treated with 10 mg/kg b.w. at 10, 30, 60 and 90 minutes postshock. There were five dogs per group. Blood pressure, PPT, platelet aggregability and lung wet/dry weight ratios were studied (I).

The endotoxin model was treated with ibuprofen, a cyclooxygenase inhibitor, 10 mg/kg b.w. either 10 minutes before or after endotoxin injection and repeated at one hour postshock. In this experiment there were four dogs per group. Blood pressure, PPT, platelet aggregability, wet/dry lung weight ratio, platelet and white blood cells counts and hematocrit were studied (III).

The trauma model was treated with naloxone 2 mg/kg b.w. either 10 minutes before trauma or 30 minutes posttrauma followed by 1 mg/kg b.w. every 30 minutes and with cyproheptadine, an antiserotonin drug, 1 mg/kg 30 and 60 minutes posttrauma. Blood pressure, PPT, platelet aggregability and wet/dry lung weight ratio were studied. There were five dogs per group (II).

In the dogs, the endorphin model was studied in conjunction with i.v. naloxone treatment 2 mg/kg b.w. 10 minutes before endorphin injection repeated thereafter every 30 minutes. Blood pressure, heart rate, pulmonary platelet trapping, wet/dry lung weight ratio, intracranial pressure, serum catecholamines, blood and CSF B-endorphin immunoreactivity, platelet and white cell counts and hematocrits were done (IV).

In the rabbits i.v. naloxone 1 mg/kg b.w. was given at the same intervals as in the dogs. Blood pressure, heart rate. PPT, plasma fibrinolytic activity, fibrin split products, plasma kallikrein inhibitor, plasma antithrombin III, plasma kallikrein like activity and in vitro platelet aggregability were done (V).

The survival study was done on the endotoxin model treated with the following drugs in various combinations: naloxone (N) 2 mg/kg at 10 minutes postshock repeated hourly for five hours, ibuprofen (I) 5 mg/kg b.w. 10 minutes postshock, methyl-prednisolone (MP) 30 mg/kg b.w. 10 minutes and five hours postshock. Furosemide, 20 mg, was given two hours postshock and normal saline was administered at a rate of 200 ml per hour for five hours. On four or more dogs in each group blood pressure was monitored for five hours and platelet, white cell counts and hematocrit were taken hourly for the first five hours. The following groups were studied:

I	Endotoxin alone	IV	Endotoxin + MP and I
II	Endotoxin + MP	V	Endotoxin + N and I
III	Endotoxin + MP and N	VI	Endotoxin + MP + N + I

COMMENTS ON MATERIAL AND METHODS

Experimental animals:

Papers I, II, III, IV and VI were done in Rochester, New York, USA. In that laboratory dogs have been used as experimental animals for over 20 years. During the last 10 years the main efforts have been to elucidate the pathophysiology of pulmonary platelet trapping. Paper V was done in Malmö, Sweden, and due to animal costs we were forced to change species. Rabbits were chosen because the same technique of injecting B-endorphin can be used as in the dogs. In addition, changing experimental animals gives information about species specific reactions.

Anesthesia:

All anesthetic drugs have cardiovascular effects. In both dogs and rats it has been shown that pentobarbital anesthesia does not produce cardiovascular changes that are naloxone reversible in non-shocked animals (26), which otherwise might have obscured the interpretation of naloxone's effect on the hypotension due to trauma and to endotoxin injection.

Tagging procedure:

Indium has been reported to be a more efficient tag for platelets than ^{51}Cr (98). However, its half live is 58 hours as compared to 28 days for ^{51}Cr . Since in our dog experiments we have dried the lungs for 5 - 7 days in order to measure wet/dry lung weight we chose ^{51}Cr as the most appropriate isotope. For the sake of consistency we also used ^{51}Cr in the rabbits although we did not dry these lungs.

To obtain an efficient tag in the rabbits we were forced to use homologous platelets, since removal of the amount of blood needed to tag platelets for each rabbit would induce shock which alters the platelets aggregability (72).

Platelet trapping and wet/dry lung weight.

We chose to count the whole lungs since we have seen that the radioactivity is not uniformly distributed between the lung lobes. The lower lobes tend to have more radioactivity than the upper lobes. The wet/dry lung weight ratio is a crude method of determining pulmonary edema. We did not use the more sensitive green-dye double indicator dilution method to measure extravascular lung water because it would have interfered with our technique for PPT measurement, the primary purpose of our studies.

Ex vivo platelet aggregation

In human plasma, ADP in concentrations 0.2 - 5 μ M can induce platelet aggregation in two phases. When the ADP concentration is increased the two phases can no longer be distinguished on the aggregation curve. The second phase of aggregation is associated with the release reaction in which ADP, serotonin and arachidonic acid derivatives such as thromboxane A_2 are released. These substances act synergistically with ADP to promote further aggregation. Platelet aggregation due to collagen is always associated with the release reaction. In order to initiate platelet aggregation Ca^{2+} ions and fibrinogen must be present, temperature must be between $+10^{\circ}C$ to $+45^{\circ}C$ and pH must be over 6.5 (80).

Drugs:

- Naloxone-HCl is an opiate receptor blocker with a serum half life of approximately 70 minutes in dogs. Animal studies have shown naloxone pharmacologically inert, and its sole action seems to lie in blocking opiates (23, 85).
- Sodium ibuprofen: When our studies with ibuprofen were instituted, the opinion of the Upjohn Co and of Jacobs et al (56) was that ibuprofen was a selective thromboxane synthetase inhibitor. It is, however, a cyclooxygenase inhibitor and blocks the formation of prostaglandins derived via this pathway. Ibuprofen is a very potent inhibitor of platelet aggregation and in high doses it also inhibits leucocyte aggregation (33, 55).

- Cyproheptadine HCl is a very potent antiserotonin and antihistamine drug; it is used in a subgroup in paper II. Its antiserotonin properties are comparable to that of lysergic acid. Cyproheptadine cannot be considered a specific antiserotonin or antihistamine agent, as it has other unspecified pharmacological effects, including anticholinergic properties (109, 110). This limits its usefulness in delineating the pathologic role of serotonin. With today's knowledge ketanserin would have been a better choice.

Endotoxin model: In this LD₁₀₀ model there is an initial dramatic drop in blood pressure which often occurs during the injection (drops to below 20 mm Hg are not uncommon). This is followed by a partial recovery and a gradual second drop. Most dogs experience diarrhea, often accompanied by blood. This model has been shown in this laboratory and by other investigators to cause a consistent and significant pulmonary platelet trapping (44). The use of endotoxin to induce shock has in recent years been questioned. Although it has been shown that live bacteria produce a lung injury similar to that seen with endotoxin (15) many favor live bacteria since such a model more closely resembles clinical sepsis. However, in these experiments we were interested in the mechanism of pulmonary platelet trapping. Intravenous injection of endotoxin induces PPT rapidly and consistently and is therefore a favorable model for these experiments. PPT in endotoxin shock is most evident in the first 30 minutes after endotoxin injection; thereafter platelets gradually reappear in the systemic circulation (44). Two hours was chosen as the time for sacrifice in the acute endotoxin model so that evidence of platelet sequestration would still be detectable in the lung, while allowing sufficient time for the drugs being studied to have an effect.

Trauma model: The trauma was administered by the same person to minimize variations in soft tissue trauma. The dogs were sacrificed after four hours, since it has been shown that the pulmonary platelet trapping shows gradual increase in traumatic shock and is detectable at this point (44). Previous studies from this laboratory show that this technique causes an initial drop in systemic blood pressure and a pulmonary platelet trapping at four hours which is significantly higher than sham controls (112). In Blalock's description he used a hammer to induce trauma, while we used a padded mallet. This offers an explanation for the less severe hypotension we obtained.

B-endorphin model: There are today two commercially available B-endorphins; human B-endorphin and camel B-endorphin. In a pilot study in dogs, human B-endorphin showed a greater immunoreactivity to CNS areas known to contain opiate receptors than did camel B-endorphin. These studies were not done in the rabbits and it is possible that camel B-endorphin might have been a better choice for rabbits since we did not see the same effects on blood pressure and on heart rate as in the dogs. However, this might also represent species differences.

A INFLUENCE OF PROSTANOIDS AND ENDORPHINS.

RESULTS

Control: In none of the experiments were there any significant changes in either the sham controls or the individual drug controls, nor between these two groups. Thus blood pressure up to four hours, PPT at two or four hours, platelet aggregability at two or four hours, wet/dry lung weight ratios at two or four hours, platelet and WBC counts up to two hours and HCT up to two hours remained unchanged.

Endotoxin shock: Endotoxin injection induced an initial significant blood pressure drop. Neither naloxone nor ibuprofen prevented this early hypotension. At two hours the late hypotension was significantly less in the dogs treated with high dose naloxone or with low dose naloxone given before shock induction (I). Ibuprofen given either before or after shock had a dramatic effect on the late hypotension (III). Naloxone caused a reduction in PPT which was significantly different from that of the endotoxin controls when the dogs were pretreated (I). Both pre and posttreatment with ibuprofen gave PPT significantly less than that seen in untreated endotoxin dogs. Ibuprofen pretreated dogs did not have PPT significantly different from shams (III).

ADP-induced platelet aggregability was increased in endotoxin shocked dogs. This increase was reduced by both naloxone and ibuprofen treatment; some treated dogs had platelet aggregability reduced to below baseline values.

The hematocrit increased significantly in all endotoxin injected dogs; pretreatment with ibuprofen showed a significantly lesser increase (III). The initial drop in both peripheral platelets and WBC was unaffected by ibuprofen treatment. The platelet recovery at two hours was significantly higher in the two ibuprofen treated groups compared to endotoxin control. The recovery of WBC was higher in the ibuprofen treated groups compared to endotoxin control but only significantly higher when ibuprofen was given as pretreatment (III).

Wet/dry lung weight ratio showed no significant difference between any of the groups.

Trauma shock model: This soft tissue trauma model caused a small but significant initial blood pressure reduction; blood pressure returned to control values by 30 minutes. This hypotension was not seen when the dogs were pretreated with naloxone.

PPT was significantly higher in the traumatized dogs compared to sham controls. This increase was not seen in any of the naloxone or cyproheptadine treated dogs, and these were not significantly different from the sham control.

Platelet aggregability was slightly increased in the traumatized dogs; treated dogs were not different from control. The wet/dry lung weight ratio was not increased in this trauma model at four hours (II).

COMMENTS

Papers I, II, III are aimed at investigating indirectly the roles of endorphins and arachidonic acid derivatives derived via the cyclooxygenase pathway, on PPT and hypotension in experimental shock models by blocking their effects with naloxone and ibuprofen. It is, however, possible that naloxone has other not yet known effects and that ibuprofen, in addition to blocking cyclooxygenase also acts on other arachidonic acid products, such as the leucotrienes.

Many animal models have been used to study experimental endotoxin shock. The dosages of endotoxin used by various investigators range from as low as 1 ug/kg b.w. up to 20 mg/kg b.w. and the resulting

injury also varies widely (13, 20). Variations in results have been attributed to species differences when in fact the investigators cited also used many sources of endotoxin or live bacteria as well as widely varying dosages (312. In the interest of consistency we have used an LD₁₀₀ endotoxin model of the same species for all our studies, and have compared the study of this very potent injury with a study of a much milder one, soft tissue trauma.

We found that the opiate antagonist naloxone significantly reduced the late hypotension of endotoxin shock when it was given as pretreatment or as a high dose postendotoxin injection. It also completely prevented the hypotension associated with soft tissue trauma. Pretreatment with naloxone did not affect the initial hypotension in the endotoxin shock model and posttreatment with the low dose was insufficient to reduce late hypotension. From these results it can be concluded that the initial hypotension in the endotoxin model is not endorphin dependent but the late hypotension and the hypotension due to soft tissue trauma may be, at least in part, endorphin mediated. PPT was significantly decreased when naloxone was given as pretreatment in the endotoxin shock model and prevented when given either before or after soft tissue trauma. Naloxone given after the induction of endotoxin shock did not significantly reduce pulmonary platelet trapping although some reduction was seen. PPT is an immediate response to endotoxin injection and giving this dosage of naloxone 10 minutes after shock induction may already be too late. In both these models the increase in ADP-induced platelet aggregability was reduced by naloxone. We found no reference in the literature concerning the effect of B-endorphin on platelet aggregability or on PPT in any shock model. It has been reported that morphine by itself does not increase platelet aggregability *in vitro* but inhibits the antiaggregating properties of prostaglandin E₁ (101). Our findings together with the findings of others (54, 125) indicate that B-endorphin is increased in different shock situations and suggest that B-endorphin may play a role in the evolution of ARDS resulting from shock and trauma.

Our experiments show that the cyclooxygenase inhibitor ibuprofen, given either before or after endotoxin shock, significantly reduces late hypotension, while it has no effect on early hypotension. PTT was also significantly reduced by ibuprofen posttreatment and not significantly different from sham controls when ibuprofen was given

as pretreatment of endotoxin shock. We found a recovery of peripheral platelets and leukocytes in both the ibuprofen treated groups compared to endotoxin controls. This effect may be attributed to ibuprofen's antiaggregating properties and also be associated with its ability to attenuate the increase in pulmonary artery pressure as reported by Demling (24). It is reasonable to assume that ibuprofen promotes the pulmonary microcirculation, which should result in improved oxygenation. Our results suggest a prominent role of arachidonic acid derivatives via the cyclooxygenase pathway in the etiology of ARDS and hypotension in septic shock.

B INFLUENCE OF CENTRALLY ADMINISTERED B-ENDORPHIN

RESULTS

Dogs: In the sham controls there was a gradual drop in heart rate beginning after 2 hours, and the mean decrease from base line at five hours was 23 ± 5 beats/minute. B-endorphin injected into the cisterna magna caused an immediate decrease in heart rate which was already significantly different from sham control by 30 minutes; the mean drop after 5 hours was 73 ± 33 beats/minute. When B-endorphin was injected into the lateral ventricle there was a slight increase in the heart rate that lasted approximately 15 minutes, followed by a progressive decrease. The drop in heart rate recorded at 5 hours did not differ significantly between these two groups. Naloxone treatment abolished the endorphin mediated changes in the heart rate (IV).

Blood pressure was stable throughout the five hours in sham controls. B-endorphin injected into the cisterna magna caused an early drop in blood pressure seen at 10 minutes and significant at 30 minutes. Blood pressure reduction caused by injection into the lateral ventricle was not evident until one hour, and was significant only by two hours. Naloxone treatment abolished the drop in blood pressure (IV).

Intracranial pressure did not change in the sham control. Injection of B-endorphin caused a slight increase (3 - 6 cm H₂O) that was not abolished by naloxone treatment (IV).

The hematocrit was unchanged throughout the experiment in all the dogs. Wet/dry lung weight ratio tended to increase in both endorphin models, but the increase was not significant compared to shams. Naloxone treated animals had normal wet/dry lung weight ratios.

Peripheral platelet count was stable throughout the experiment in all groups.

Peripheral WBC count was stable in all groups except the intravenicularly injected group which showed an increase by five hours. None of the dogs showed any change in epinephrine, norepinephrine or dopamine compared to shams.

PPT was increased in both untreated B-endorphin injected groups. This increase was significant when B-endorphin was injected into the lateral ventricle. Naloxone pretreated animals had normal PPT.

Endotoxin caused a marked increase in circulating B-endorphin, while there was a decrease in the cerebrospinal fluid.

Rabbits: Blood pressure and heart rate were stable throughout the observation period in the control group. B-endorphin caused a slight but insignificant early drop in blood pressure but had no effect on heart rate.

The ^{51}Cr lung/blood ratio was significantly higher in the B-endorphin injected rabbits. Fibrinolytic activity measured on fibrin plates showed no significant differences between the groups, but there was a decrease in the group that received both B-endorphin and naloxone. There were no measurable fibrin split products in any of the groups.

Antithrombin III, kallikrein like activity and kallikrein inhibitor activity did not differ significantly between the groups. The in vitro platelet aggregability in response to ADP and collagen did not differ significantly between any of the groups.

COMMENTS

Holaday and Faden were the first to propose that the endogenous opiates are involved in the pathophysiology of shock. In a rat

endotoxin model they showed naloxone effective in reducing hypotension (55). In follow-up studies they showed similar positive effects of naloxone in hemorrhagic shock in rats, and in both endotoxin and hemorrhagic shock in dogs (26). In these studies they showed an increased survival time in both rats and dogs subjected to hemorrhagic shock and in dogs subjected to endotoxin shock. These results were later confirmed by other investigators who, in addition, showed that naloxone treated endotoxin shocked dogs had less severe acidosis and hypoglycemia (22, 37, 93, 100). Increased amounts of circulating B-endorphin have been shown in rats, monkeys, and dogs subjected to endotoxin shock (54, 125, IV), in rats subjected to trauma (36) and in dogs subjected to hemorrhagic shock (66). The source of this B-endorphin seems to be the pituitary gland since no increase was seen in hypophysectomized animals (36). These findings indicate that the plasma endorphin levels are increased in shock and may have deleterious effects.

The site of action of the endorphins is still under investigation. Most investigators favor a central action (54). It has been shown that small amounts of naloxone, which are ineffective when given i.v., protect against hypotension in endotoxin shocked dogs when injected into the lateral ventricle (59). In other experiments including our own, B-endorphin, injected centrally in amounts that are ineffective when given systemically, produce hypotension and bradycardia (IV, 67, 82). It is tempting to interpret this as a central action. However, alternative explanations are possible: naloxone may, when injected centrally, block release of B-endorphin from the pituitary, and conversely, small amounts of B-endorphin when given centrally may trigger release of additional B-endorphin from the pituitary.

B-endorphin injected intraarterially into an isolated stomach segment caused injury to the epithelial cells similar to that seen when live bacteria were injected via the same route. Naloxone prevented this cell injury (90). In a recent article Albert et al showed naloxone protective against muscle cell injury in hemorrhagic shocked rats even when the blood pressure was kept low (3). These findings together with the findings of opiate receptors outside the CNS (82), indicate that B-endorphin may also have peripheral effects.

Our studies do not offer any explanation for mechanism by which B-endorphin causes PPT. It could act by releasing humoral factors

other than those studied and/or it could act on nervous mechanisms. We favour the latter which agrees with the findings that centrally administered endorphin decreases sympathetic outflow and increases vagal tone (54) as well as the finding that denervation of the lung is protective against PPT (116).

C INFLUENCE OF PROSTANOID AND ENDORPHIN BLOCKERS ON SURVIVAL IN ENDOTOXIN SHOCK.

RESULTS

The initial blood pressure decrease recorded by 10 minutes post shock induction was similar in all groups. In the untreated dogs pressure stayed low throughout the five hour period. The dogs treated with methylprednisolone did not differ significantly from endotoxin controls. When naloxone was also added the blood pressure drop at five hours was significantly less compared to controls. Dogs in the ibuprofen treated groups had a fast recovery of blood pressure, by 30 minutes post shock it was already significantly higher than controls, and above control values at five hours.

Hematocrit rose in all groups with no significant differences between the groups at five hours.

The initial drop in circulating platelets and leukocytes was similar in all the groups. The dogs treated with ibuprofen and methylprednisolone had a significantly greater drop of platelets compared to control at five hours, while the other treated groups were not significantly different compared to controls.

The percent drop in circulating leukocytes recorded at five hours was significantly less only in the group treated with naloxone, ibuprofen and methylprednisolone, compared to that of the control groups.

The incidence of bloody diarrhea was less in all the treated groups compared to controls; 100 % in the control group and 10 - 20 % in the treated groups.

Untreated endotoxin shocked dogs all died within 16 hours. I.v. infusion of fluids, diuretics, periodic hyperinflation of the lungs and

turning the animals did not prolong survival beyond 30 hours. Methylprednisolone alone did not prolong survival time. When naloxone was added to the methylprednisolone treatment it delayed mortality. The group treated with ibuprofen and methylprednisolone had three permanent survivors of nine and there were five permanent survivors of nine dogs treated with naloxone, ibuprofen and methylprednisolone in combination.

COMMENTS

Septic shock is a major complication of abdominal surgery, trauma and burns (65, 68, 123). It is associated with multi-organ failure and if it is not reversed at an early stage it evolves into an irreversible state ultimately resulting in death (42, 70). Although the use of respiratory assistance with positive end expiratory pressure, steroids, broad spectrum antibiotics and carefully titrated fluid resuscitation combined with earlier detection has improved survival, septic shock is still associated with high mortality rates (40 - 70 %) (65, 68, 123).

The pathophysiology of septic shock is still unsolved and under considerable debate. The shock state induces the activation of several cascade systems such as the complement, the coagulation and the fibrinolytic systems (9, 42, 70, 79). The adrenals are stimulated with consequent increase of plasma catecholamines and steroids (69). Increased amounts of circulating histamine, bradykinin, and prostaglandins are also seen (19, 29, 34, 53).

In recent years the endorphins have been shown to increase during shock and are suggested as contributors to the deleterious effects of shock, since blocking with naloxone, an opiate antagonist, has been shown to be beneficial in both hemorrhagic and endotoxic shock (26).

Acidosis is often present in septic shock and reflects the impaired tissue oxygenation resulting in utilization of anaerobic metabolism. Damage to the kidneys results in oliguria and accumulation of toxic substances in the circulation. Ischemic intestine has been shown to release substances that depress the myocardium and this further contributes to the state of hypoperfusion (27, 39, 71, 75).

The treatment of septic shock, in addition to antibiotics and fluids, must be directed not only to increasing the blood pressure but also to improving the circulation in the microvasculature of the affected organs. Simply increasing the blood pressure by treatment with catecholamines has been shown inadequate for this purpose and may even cause further damage by increasing vasoconstriction (69, 123). We have seen, in pilot studies on endotoxin shocked dogs, that norepinephrine in amounts sufficient to increase blood pressure caused an increase in PPT. The use of α -adrenergic blockers is helpful in improving microcirculation but presents difficulties because of their hypotensive effects (42).

The use of respiratory assistance with positive end expiratory pressure (PEEP) is currently being advocated as an important aspect of ARDS therapy (61, 103, 121), but can only be truly effective when combined with measures which improve the microcirculation.

Although there is much controversy concerning the usage of steroids in septic shock, steroids are known to have pharmacological effects which indicate a theoretic usefulness in this condition. It has been proposed that steroids enhance endothelial integrity, particularly in the capillaries, interfere with degranulation of polymorphonuclear leukocytes, presumably by stabilization of lysosomal membranes, have vasodilatory effects, probably by stimulating B-adrenergic receptors, have anticoagulating effects, inhibit complement activation induced by endotoxin, decrease the serum levels of antidiuretic hormone (ADH) and reduce edema of the cerebral nervous system (10, 38, 41, 46, 69, 105, 106, 122).

Considering all these positive properties one would expect a dramatic increase in survival of endotoxin shocked animals treated with steroids. However, this has not been shown to be the case; while in some studies survival rate is improved, the overall results are disappointing (4, 18, , 50, 51, 52). In models similar to ours the most positive results have been presented by Hinshaw et al who showed an increase in survival time but no permanent survivors beyond twenty-four hours (120).

Naloxone is another possible candidate for use in the attempt to increase survival rate in both hemorrhagic and endotoxic shock. Endorphin released by shock causes hypotension and bradycardia (54). When B-endorphin is injected centrally in dogs and rabbits it causes PPT (IV, V). The blocking of these effects by the opiate antagonist naloxone might be expected to improve survival of endotoxin shocked animals. However, again the results in such studies are not impressive, and when high doses of naloxone were used to treat an LD₁₀₀ endotoxin shock model in dogs there was only a 20 % increase in "permanent" survivors to 96 hours (93).

To date there have not, to our knowledge, been any survival studies for ibuprofen treatment of shock, but ibuprofen has been shown to increase blood pressure, and cardiac performance, and to attenuate acidosis in canine endotoxin shock (58). These findings taken together with our own finding, that it decreases PPT (III), makes it likely that ibuprofen might improve survival in endotoxin shock.

The result of the combined treatment with naloxone, ibuprofen and methylprednisolone on survival is the most impressive reported in experimental studies using a model similar to ours. It also focuses attention on pharmacological treatment as an adjuvant to traditional shock therapy. It is important to emphasize when discussing this kind of treatment, that it should be used only as an adjuvant to generally accepted resuscitation management.

GENERAL DISCUSSION

The lung injury secondary to sepsis, trauma and burns is clinically characterized by noncardiac pulmonary edema; by reduction of PaO₂, often refractory to increased oxygen delivery; and by decreased pulmonary compliance. The histologic picture consists of edema, fibrosis and the presence of a large number of microemboli in the small pulmonary arterial branches and capillaries (7, 17, 92, 110, 119).

The evolution of clinical ARDS has been divided by Moore into four phases. Phase I is the initial pulmonary response to trauma believed to occur as a constant phenomenon. During phase 2 there is normalization of the pulmonary reaction. Phase 3 develops in only a few cases and is characterized by hypoxia and interstitial pulmonary

edema. Phase 4 is cardio-respiratory collapse (81). From animal studies it has been proposed by Smith et al. that the lung injury takes place in two phases. Phase I is characterized by trapping of platelets and leukocytes with a concomitant increase in pulmonary artery pressure and an increase in protein poor lymph flow. In phase II there is normalization of the pulmonary artery pressure and return of platelets to the circulation, but lymph flow is still high and is protein rich, indicative of pulmonary endothelial damage (108). In these studies the composition of the lung lymph is assumed to be representative of the net flux of water, electrolyte and proteins transversing the pulmonary endothelium (15).

Many factors have been suggested as mediators of the pulmonary damage seen in ARDS such as PPT, pulmonary trapping of leukocytes, activation of the complement system, accumulation of fibrin degradation products in the pulmonary vasculature, and release of the vasoactive substances serotonin, histamine, arachidonic acid derivatives and bradykinin (6, 7, 14, 40, 63, 64, 84, 90, 112).

In both sepsis and trauma there is an accumulation of platelets in the lung that correlates with disappearance of platelets from the circulation (44, 45, 85). In sepsis this is an immediate response with normalization already beginning by 30 minutes, while in trauma this is a more gradual process happening over many hours (44, 45). This difference may contribute to the higher potency of the endotoxin model. It has been shown that injected endotoxin is picked up by platelets and that the resulting complex is sequestered in the lung (11, 72). Vaage et al have shown that platelet embolization in the lung causes an increase in vascular permeability (117).

Both sepsis and trauma increase platelet aggregability (72) and when platelets aggregate they undergo a release reaction (80). During this process ADP and serotonin are released. Arachidonic acid derivatives including is thromboxane A_2 are formed and released into the circulation (76, 103). These released substances are potent platelet aggregators and promote further aggregation (78) and consequently more PPT. In addition, both serotonin and TxA_2 are strong vasoconstrictors and are thought to contribute to the increase in pulmonary artery pressure, since this increase can be attenuated when inhibitors of either serotonin or TxA_2 are used to treat experimental shock models (62, 112). Myrvold found that making

dogs thrombocytopenic attenuated the increase in pulmonary artery pressure seen with injection of disintegrated pseudomonas, but had no effect on the pulmonary trapping of leukocytes. Neither was there any effect on the pulmonary endothelial damage, which focused attention on leukocytes as possible mediators of the pulmonary injury in sepsis (85). When microembolisation was induced in leukocytopenic sheep the same initial rise in pulmonary artery pressure was seen as in normal sheep, but the increased vascular permeability to proteins (Phase II) was attenuated (111). Craddock has shown that activation of complement is necessary for the leukocytes' ability to induce endothelial cell damage in vitro (21). Several investigators have shown that endotoxin and trauma activate the complement system (1, 30, 44, 45, 56). When the complement system is activated by cobra venom factor in rabbits there is sequestration of leukocytes in the lung but no sign of endothelial damage. If, however, hypoxia is induced in the same model endothelial damage is seen (47). It has been shown in experiments using ADP injection in sheep that hypoxia is obviated if the animals are made thrombocytopenic (76).

When Brown injected an LD₁₀₀ endotoxin dose into rabbits the platelets disappeared from the circulation, but when the same dose was injected into C₃ - C₉ complement depleted rabbits the platelet counts were normal and all animals survived (12). These observations taken together imply a role for both platelets and leukocytes in the pulmonary damage and in the outcome of a lethal dose of endotoxin. Furthermore, Jacob showed in an in vitro model that the capability of complement activated leukocytes to destroy endothelial cells is significantly augmented when either platelets or serotonin are present (57). The leukocytes probably destroy endothelial cells by releasing oxygen derived free radicals (98, 124).

Serotonin by itself, when injected into the pulmonary circulation in dogs, causes a pulmonary injury similar to that seen in ARDS, and pretreatment of a hemorrhagic shock model with methysergide, an antiserotonin agent prevented lung injury in dogs (63). The chief sources of serotonin are unstable platelets and hypoxic intestine both of which are present in septic shock (63, 72).

Elevated histamine levels have also been demonstrated in endotoxin shock (118), and in a recently published article injection of histamine into sheep caused an increase in capillary leakage of proteins (14).

Various arachidonic acid derivatives are increased in shock (60, 108). TxA_2 and prostacyclin (PGI_2) are of special interest because of their ability to modulate platelet and leukocyte behavior (109). TxA_2 is produced in platelets, lung parenchymal cells and intestinal cells and is one of the most powerful platelet aggregators; it also aggregates leukocytes and is a very potent vasoconstrictor (101). PGI_2 , which is mainly produced in endothelial cells, has the opposite effects (76). During normal circumstances these two substances seem to balance each other, but in septic shock, while both are increased, the increase of TxA_2 is relatively greater (28). This may contribute to platelet and leukocyte aggregation and to the increase in pulmonary artery pressure.

When PGI_2 is injected before the onset of endotoxin shock in sheep it both attenuates the increase in pulmonary artery pressure and decreases the leakage of proteins (25). If imidazole, a selective TxA_2 synthetase inhibitor, is injected into endotoxin shocked sheep the same positive effects are seen (62). When indomethacin, a cyclooxygenase inhibitor, is used to treat endotoxin shocked sheep the pulmonary artery pressure increase is attenuated but leakage of proteins (Phase II) is augmented (87). This is attributed to the fact that indomethacin blocks both the formation of TxA_2 and PGI_2 (101). In these experiments neither the initial decrease in platelets nor the initial decrease in leukocytes was affected, indicating that it is not only the mechanical plugging of the microvasculature by platelets and leukocytes that does the damage but also, and perhaps more importantly, release of various substances from these cell types. This was proposed in 1971 by R  degran et al who showed that the increase in pulmonary artery pressure in dogs subjected to protamine injection was inhibited if the dogs were pretreated with ASA (97).

Bradykinin is another vasoactive substance increased in shock. It is normally metabolized by angiotension converting enzymes in the lung; hypoxia inhibits the activity of this enzyme, and during hypoxic circumstances bradykinin increases the microvascular permeability to proteins in the lung (90).

Fibrin and its degradation products have also been implicated as mediators of the lung injury. Saldeen found that by defibrinogenating dogs before thrombin and tranexamic acid were injected lung

injury was prevented. Blocking of vasoactive substances or complement depletion did not prevent the injury caused by thrombin and tranexamic acid (101). In another experiment, fragment D, a purified human fibrinogen degradation product, injected into rabbits increased ADP- induced platelet aggregability and resulted in ARDS. This response was not seen in thrombocytopenic rabbits (77).

Many of the features observed in the animal models described above also apply in the case of humans with ARDS. In this situation there is an increase in pulmonary artery pressure (35), decrease in PaO_2 (35), complement activation (40), disappearance of platelets and leukocytes from the circulation (43, 104), pulmonary edema (96), and roentgenologic evidence of pulmonary microthrombi (35).

In summary, ARDS presents with an increase in pulmonary artery pressure accompanied by increased plasma B-endorphin, increased plasma TxA_2 , decrease in circulating platelets and leukocytes, and increase in pulmonary platelet and leukocyte trapping resulting in endothelial damage in the lung. While there is a current tendency in discussion of the etiology of ARDS to deemphasize the role of the platelets, there is considerable evidence that both the platelets and leukocytes are important in the evolution of this condition and that their roles may be interdependent.

CLINICAL IMPLICATIONS

It is always difficult to extrapolate findings in experimental animals to man, not only because of species differences but also because it is difficult to mimic the clinical situation in the laboratory. This is true both for live bacteria and endotoxin models when we try to explore the pathophysiologic events in septic shock.

Naloxone has already been reported to improve hemodynamic parameters in patients with different kinds of septic shock (47, 87, 111), which implies that opiates are also involved in the pathophysiology of septic shock in man. From animal studies we know that simultaneous administration of opiate analgesics and endotoxin or live bacteria exacerbate the hypotension (52). This finding, taken together with the finding that naloxone has beneficial effects in shock, leads to a warning against using opiate analgesics in shock and instead promotes the use of regional anesthesia.

Naloxone also has the obvious disadvantage of blocking opiate pain receptors, which makes it difficult to use in clinical practice. However, recent data from experimental animals treated with the more selective opiate receptor blockers indicate that hypotension and analgesia may be mediated by different opiate receptors (52). It is therefore possible that in the near future we will have a selective opiate antagonist that blocks only the deleterious effects of endorphins without affecting their analgesic action. Conversely an opiate analgesic that operates only on opiate pain receptors may be found.

Ibuprofen has not yet been tried in clinical sepsis but its positive effects in experimental shock makes it a possible candidate in the future treatment of sepsis. It is possible that a selective TxA_2 inhibitor would be even more effective.

Steroids as treatment in sepsis are still controversial although they are widely used. In addition to the positive effects discussed earlier they also have a negative effect on the leucocytes' ability to phagocytize. This may explain why there are such contradictory reports on the effect of steroid treatment in shock situations, since this negative effect may predominate if they are given late in the shock state. It is therefore proposed that steroids should be used early in shock and only over a 24 hour period (46).

CONCLUSIONS

- Naloxone has been shown to significantly reduce PPT and hypotension in a canine LD_{100} E.coli endotoxin shock model and to obviate blood pressure fall and PPT in a canine trauma model, indicating that opiod peptides participate in the pathophysiology of shock and trauma.
- Centrally administered B-endorphin caused significant blood pressure and heart rate reduction in dogs and significant PPT, both in dogs and rabbits, substantiating the above findings.
- Ibuprofen, a cyclooxygenase inhibitor, to significantly decreased PPT and hypotension in a canine LD_{100} E.coli endotoxin shock model indicating a prominent role for prostaglandins derived via the cyclooxygenase pathway in the pathophysiology of endotoxin shock.

- Combined treatment with naloxone, methylprednisolone and ibuprofen significantly increased the number of permanent survivors in a canine LD₁₀₀ E.coli endotoxin shock model, indicating this combination as a possible adjunct to traditional shock therapy.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all who have inspired and supported the study in particular:

Associate Professor Ulf Haglund and Professor Seymour I Schwartz my teachers in research.

Professor Karl M Knigge for stimulating and constructive collaboration.

Professor Bengt Zederfeldt for constructive criticism and support.

Professor Sven-Erik Bergentz and Associate Professor David Bergqvist for valuable help and criticism in preparing the manuscript.

Professor Bo Eklöf who gave me the opportunity to start in research.

All my colleagues in the Department of Surgery and Urology, Helsingborg County Hospital.

My co-workers in research.

Ms June Ljungberg, Mr Roger Bender and Mr Ulf Stjärnqvist for skilled technical assistance.

Mrs Anita Wallin Gjöres, Ms Eva Pral and Ms Janice Black for skilled secretarial help.

Marian Kuenzig, B.S., for invaluable help.

This study was supported by grants from National Institute of Health (Grant NO HL 13466), Swedish Medical Research Council (proj.no. 04502), the Medical Faculty, University of Lund and Thorsten and Elsa Segerfalks Foundation for Medical Research and Education.

REFERENCE LIST

- 1 Aasen AO, Mellbye OJ, Ohlsson K: Complement activation during subsequent stages of canine endotoxin shock. *Scand J Immunol* 8:509-513, 1978.
- 2 Abrahamsen AF: A modification of the technique for ^{51}Cr labelling of blood platelets giving increased circulatory radio-activity. *Scand J Haematol* 5:53-63, 1968.
- 3 Albert SA, Shires III GT, Illner H, Shires GT: Effects of naloxone in haemorrhagic shock. *Surg Gynecol Obstet* 155:326-332, 1982.
- 4 Balis JU, Paterson JF, Shelley SA, Larson CH, Fareed J, Gerber LJ: Glucocorticoid and antibiotic effects on hepatic microcirculation and associated host responses in lethal Gram-negative bacteremia. *Lab Invest* 40:55-65, 1979.
- 5 Bergentz S-E, Lewis DH, Ljungqvist U: Trapping of platelets in the lung after experimental injury: Sixth Europ Conf Microcirculation. *Basel, Krager S*: 34-40, 1971.
- 6 Bergentz S-E, Ljungqvist U, Lewis DH: The distribution of platelets, fibrin and erythrocytes in various organs following thrombin infusion: An experimental study in dogs with and without antifibrinolytic therapy. *Surgery* 71:190-195, 1972.
- 7 Blaisdell FW, Lim Jr RC, Stallone RJ: The mechanism of pulmonary damage following traumatic shock. *Surg Gyn Obstet* 130:15-22, 1970.
- 8 Blalock A: Experimental shock. *Arch Surg* 20:959-996, 1930.
- 9 Bone RC, Francis PB, Pierce AK: Intravascular coagulation associated with the adult respiratory distress syndrome. *Am J Med* 61:585-589, 1976.
- 10 Bowen JM: Are corticosteroids useful in shock therapy. *JAVMA* 177:453-455, 1980.

- 11 Bredenberg CE, Taylor GA, Webb WR: The effect of thrombocytopenia on the pulmonary and systemic hemodynamics of canine endotoxin shock. *Surgery* 87:59-68, 1980.
- 12 Brown DL, Lachmann PJ: The behavior of complement and platelets in lethal endotoxin shock in rabbits. *Int Arch Allergy* 45:193-205, 1973.
- 13 Brigham KL, Bowers RE, Haynes J: Increased lung vascular permeability caused by *Escherichia coli* endotoxin. *Circ Res* 45:292-297, 1979.
- 14 Brigham KL, Owen PJ: Increased sheep lung vascular permeability caused by histamine. *Circ Res* 37:647-657, 1975.
- 15 Brigham KL, Woolverton W, Blake L, Staub N: Increased sheep lung vascular permeability caused by *Pseudomonas* bacteremia. *J Clin Invest* 54:792-904, 1974.
- 16 Busch C, Saldeen T: Amount of fibrin in different organs after intravenous, intraportal and intraaortal injection of thrombin in the rat. *Thrombos Diathes Hemorrh* 29:87-93, 1973.
- 17 Clowes GHA, Hirsch E, Williams L, Kwasnik E, O'Donnell TF, Cuevas P, Saini VK, Moradi J, Ferizan M, Saravis C, Stone M, Kuffler J: Septic lung and shock lung in man. *Ann Surg* 181:681-692, 1975.
- 18 Coalson JJ, Benjamin BA, Archer LT, Beller BK, Spaet RH, Hinshaw LB: A pathologic study of *Escherichia coli* shock in the baboon and the response to adrenocorticosteroid treatment. *Surg Gyn Obstet* 147:726-736, 1978.
- 19 Collier JG, Herman AG, Vane JR: Appearance of prostaglandins in the renal venous blood of dogs in response to acute, systemic hypotension by bleeding or endotoxin. *J Physiol London* 230:19-20, 1973.
- 20 Cook JA, Wise WC, Halushka PV: Elevated thromboxane levels in the rat during endotoxin shock. *J Clin Invest* 65:227-230, 1980.

- 21 Craddock PR, Hammerschmidt DE, Moldow CF, Yamada O, Jacob HS: Complement: Possible role in leukocyte margination, microvascular occlusion and endothelial damage. *Sem Hematol* 16:140-147, 1979.
- 22 Curtis MT, Lefer AM: Protective actions of naloxone in haemorrhagic shock. *Am J Physiol* 8:H416-H421, 1980.
- 23 Dashwood MR, Feldberg W: Release of opioid peptides in anaesthetized cats? *Br J Pharmacol* 68:697-703, 1980.
- 24 Demling RH: Role of prostaglandins in acute microvascular injury. *Ann NY Acad Sci* 384:517-533, 1982.
- 25 Demling RH, Smith MS, Gunter R, Gee M, Flynn J: The effect of prostacyclin infusion on endotoxin-induced lung injury. *Surgery* 89:257-263, 1981.
- 26 Faden AJ, Holaday JW: Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. *J Inf Dis* 142:229-238, 1980.
- 27 Falk A, Myrvold HE, Lundgren O, Haglund U: Mucosal lesions in the feline small intestine in septic shock. *Circ shock* 9: 27-35, 1982.
- 28 Feuerstein N, Ramwell PW: Effects of endotoxin on prostaglandin release from rat lung. *Br J Pharmac* 73:511-516, 1981.
- 29 Fletcher JR: The role of prostaglandins in sepsis. *Scand J Infect Dis*, suppl 31:55-60, 1982.
- 30 From AHL, Gewurz H, Gruninger RP, Pickering RJ, Spink WW: Complement in endotoxin shock: Effect of complement depletion on the early hypotensive phase. *Infect Immun* 2:38-41, 1970.
- 31 Genell S, Heideman M, Leandroer L, Ljungqvist U: The platelet and fibrinogen reactions in pseudomonas exotoxin shock. *Eur Surg Res* 5:321-332, 1973.

- 32 Gilbert RP: Mechanism of the hemodynamic effects of endotoxin. *Physiol Rev* 40:245-279, 1960.
- 33 Goldstein RE, Davenport NJ, Capurro NL, Lipson LC, Bonow RO, Shulman NR, Epstein SE: Relative effects of sulfinpyrazone and ibuprofen on canine platelet function and prostaglandin-mediated coronary vasodilation. *J Cardiovasc Pharmacol* 2:399-409, 1980.
- 34 Grant JA, Dupree E, Goldman AS, Schultz DR, Jackson AL: Complement-mediated release of histamin from human leucocytes. *J Immunol* 114:1101-1106, 1975.
- 35 Greene R, Zapol M, Snider MT, Reid L, Snow R, O'Connell RS, Novelline RA: Early bedside detection of pulmonary vascular occlusion during acute respiratory failure. *Am Rev Respir Dis* 124:593-601, 1981.
- 36 Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F: B-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 1367-1369, 1977.
- 37 Gurli NJ, Vargish T, Reynolds DG, Lechner RB: Opiate receptors and endorphins in the pathophysiology of hemorrhagic shock. *Surgery* 89:364-369, 1981.
- 38 Haglund U, Hellman A, Häggendahl E, Häggendahl J, Lundberg D: Effects of adrenergic human and ganglion blockers on hemodynamics and plasma catecholamine levels after corticosteroids during haemorrhagic shock in the dog. *Acta Anaesth Scand* 4:444-450, 1980.
- 39 Haglund U, Lundgren O: Non-occlusive acute intestinal vascular failure. *Br J Surg* 66:155-158, 1979.
- 40 Hammerschmidt DE, Weaver LJ, Hudson LD, Craddock PR, Jacob HS: Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome. *Lancet* 947-949, 1980.

- 41 Hammerschmidt DW, White JG, Craddock PR, Jacob HS: Corticosteroids inhibit complement induced granulocyte aggregation: A possible mechanism for their efficacy in shock states. *J Clin Invest* 63:798-803, 1979.
- 42 Hardaway III RM: Endotoxemic shock. *Dis Col Rect* 23:597-604, 1980.
- 43 Hechtman HB, Lonergan EA, Shepro D: Platelet and leukocyte interactions in patients with respiratory failure. *Surgery* 83:155-163, 1978.
- 44 Heideman M, Kaijser B, Gelin L-E: Complement activation early in endotoxin shock. *J Surg Res* 26:74-78, 1979.
- 45 Heideman M, Kaijser B, Gelin L-E: Complement activation and hematologic, hemodynamic and respiratory reactions early after soft tissue injury. *J Trauma* 18:696-700, 1978.
- 46 Hellman A, Häggendal E, Lindfors M, Lundberg D: Haemodynamic effects of different corticosteroids in controlled haemorrhagic shock in the dog. *Acta Anaesth Scand* 22:314-322, 1978.
- 47 Henson PM, Larsen GI, Webster RO, Mitchell BC, Goins AJ, Henson JE: Pulmonary microvascular alterations and injury induced by complement fragments: synergistic effect of complement activation, neutrophil sequestration and prostaglandins. *Ann N Y Acad Sci* 384:287-300, 1982.
- 48 Hess ML, Manson NH: The paradox of steroid therapy: Inhibition of oxygen free radicals. *Circ Shock* 10:1-5, 1983.
- 49 Higgins TL, Sivak ED: Reversal of hypotension with naloxone. *Cleveland Clinic Quarterly* 48:283-288, 1981.
- 50 Hinshaw LB, Archer LT, Beller-Todd BK, Coalson JJ, Flournoy DJ, Passey R, Benjamin B, White GL: Survival of primates in LD₁₀₀ septic shock following steroid/antibiotic therapy. *J Surg Res* 28:151-170, 1980.

- 51 Hinshaw LB, Beller BK, Archer LT, Flournoy DJ, White GL, Philips RW: Recovery from lethal *Escherichia coli* shock in dogs. *Surg Gyn Obstet* 149:545-553, 1979.
- 52 Hinshaw LB, Coalson JJ, Benjamin BA, Archer LT, Beller BK, Kling OR, Hasser EM, Phillips RW: *Escherichia coli* shock in the baboon and the response to adrenocorticosteroid treatment. *Surg Gyn Obstet* 147:345-357, 1978.
- 53 Hirsch EF, Nakajima T, Oshima G, Erdös EG, Herman CM: Kinin system responses in sepsis after trauma in man. *J Surg Res* 17:147-153, 1974.
- 54 Holaday JW: Cardiovascular effects of endogenous opiate systems. *Ann Rev Pharmacol Toxicol* 23:541-594, 1983.
- 55 Holaday JW, Faden AJ: Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. *Nature* 275:450-451, 1978.
- 56 Hugli TE, Müller-Eberhard HJ: Roles of C3a and C5a in inflammation and acute shock. *Adv Immunol* 26:44-48, 1978.
- 57 Jacob HS, Moldow CF, Flynn PJ, Weisdorf DJ, Vercellotti GM, Hammerschmidt DE: Therapeutic ramifications of the interaction of complement, granulocytes, and platelets in the production of acute lung injury. *Ann NY Acad Sci* 384:489-495, 1982.
- 58 Jacobs ER, Soulsby ME, Bone RC, Wilson FJ jr, Hiller FC: Ibuprofen in canine endotoxin shock. *J Clin Invest* 70:536-541, 1982.
- 59 Janssen HF, Lutherer LO: Ventrilocisternal administration of naloxone protects against severe hypotension during endotoxin shock. *Brain Res* 194:608-612, 1980.
- 60 Kessler E, Huges RC, Bennett EN, Nadela SM: Evidence for the presence of prostaglandin-like material in the plasma of dogs with endotoxin shock. *J Lab Clin Med* 81:85-94, 1973.

- 61 Kirby RR, Downs JB, Civetta JM, Modell JH, Dannemiller FJ, Klein EF, Hodges M: High level positive end expiratory pressure in acute respiratory insufficiency. *Chest* 67:156-163, 1975.
- 62 Krausz MM, Utsunomiya T, Dunham B, Valeri CR, Shepro D, Hechtman HB: Inhibition of permeability edema with imidazole. *Surgery* 92:299-308, 1982.
- 63 Kusajima K, Ozdemir JA, Webb WR, Wax SO, Parker FB: Role of serotonin and serotonin antagonists on pulmonary hemodynamics and microcirculation in hemorrhagic shock. *J Thorac Cardiovasc Surg* 67:908-914, 1974.
- 64 Kux M, Coalson JJ, Massion WH, Guenter CA: Pulmonary effects of E.coli endotoxin: Role of leukocytes and platelets. *Ann Surg* 175:26-34, 1972.
- 65 Landesman SH, Gorbach SL: Gram negative sepsis and shock. *Orth Clin N Am* 9:611-625, 1978.
- 66 Lang RE, Bruckner UB, Herman K, Kempf B, Rascher W, Sturm V, Unger T, Ganten D: Effect of hemorrhagic shock on the concomitant release of endorphin and enkephalin-like peptides from the pituitary and adrenal gland in the dog. *Advances in Biochemical Psychopharmacologi*. E Costa, M Trabucchi. 33:363-369, 1982.
- 67 Laubie M, Schmitt H, Vincent M, Remond D: Central cardiovascular effects of morphinomimetic peptides in dogs. *Eur J Pharmacol* 46:67-71, 1977.
- 68 Ledingham JM: Septic shock. *Br J Surg* 62:777-780, 1975.
- 69 Lillhei RC, Dietzman RH, Motsay GJ, Beckman CB, Romero LH, Shatney CH: Growth of the concept of shock and review of present knowledge. *Steroids and Shock*, Baltimore, University Park Press:377-409, 1974.
- 70 Lillehei RC, Longerbeam JK, Bloch JH, Manax WG: The nature of irreversible shock: Experimental and clinical observations. *Ann Surg* 160:682-710, 1964.

- 71 Lillehei RC, Maclean LD: The intestinal factor in irreversible endotoxin shock. *Ann Surg* 148:513-525, 1958.
- 72 Ljungqvist U: Platelets in shock and trauma. *J Surg Res* 15:132-162, 1973.
- 73 Ljungqvist U, Bergentz S-E, Leandroer L: Platelet adhesiveness and aggregability after acute haemorrhage in the dog. *Acta Chir Scand* 137:1-6, 1971.
- 74 Ljungqvist U, Schwartz SI: Pulmonary trapping during shock and pulmonary embolism. *J Surg Res* 18:559-565, 1975.
- 75 Lundgren O, Haglund U: The pathophysiology of the intestinal countercurrent exchanger. *Life Sciences* 25:1411-1422, 1978.
- 76 Malik AB, Johnsen A, Tahamant MV: Mechanism of lung vascular injury after intravascular coagulation. *Ann NY Acad Sci* 384:213-234, 1982.
- 77 Manwaring D, Currei PW: Platelet mediation of fragment D-induced respiratory distress syndrome. *Surg Forum* 31:242-243, 1980.
- 78 Marcus AJ: The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. *J Lipid Res* 19:793-826, 1978.
- 79 Miller HA, Oaks WW: Therapy for septic shock. *Frontiers in Medicine* 7:26-32, 1977.
- 80 Mills DCB: The basic biochemistry of the platelet. *Haemostasis and Thrombosis*. Ed.: Bloom Aland Thomas DP. 50-60, 1981.
- 81 Moore FD: Pulmonary insufficiency - Discussion. *J Trauma* 8:666-675, 1968.
- 82 Moss JR, Scarpelli EM: B-endorphin central depression of respiration and circulation. *J Appl Physiol* 50:1011-1016, 1981.

- 83 Müller-Berghaus G: The role of platelets, leukocytes and complement in the activation of intravascular coagulation by endotoxin. Platelets: A multidisciplinary approach. G de Gaetano and S Garattini. Raven Press: 303-319, 1978.
- 84 Myrvold HE, Brandberg A, Lindqvist O, Busch C, Lewis DH: The role of platelets and fibrinogen in experimental septic shock. *Acta Chir Scand* 143:131-137, 1977.
- 85 Myrvold HE, Svalander C: Pulmonary microembolism in early experimental septic shock. *J Surg Res* 23:65-74, 1977.
- 86 Neidle A, Manigault I, Wajda IJ: Distribution of opiate-like substances in rat tissues. *Neurochem Res* 4:399-410, 1979.
- 87 Ogletree ML, Brigham KL: Indomethacin augments endotoxin induced lung vascular permeability in sheep. *Am Rev Respir Dis* 119 (suppl):383, 1979.
- 88 Oyama T, Toyooka K, Sato Y, Kondo S, Kudo T: Effect of endotoxic shock on renal and hormonal functions. *Canad Anaesth Soc J* 25:380-391, 1978.
- 89 Pace NL, Parrish RG, Lieberman MM, Wong KC, Blatnick RA: Pharmacokinetics of naloxone and naltrexone in the dog. *J Pharmacol Ext Ther* 208:254-256, 1979.
- 90 Pang LM, O'Brodivich HM, Mellins RB, Stalcup SA: Bradykinin-induced increase in pulmonary vascular permeability in hypoxic sheep. *J Appl Physiol* 52:370-375, 1982.
- 91 Peters WP, Johnson MW, Friedman PA, Mitch WE: Pressor effect of naloxone in septic shock. *Lancet* 529-532, 1981.
- 92 Pratt PC, Vollmer RT, Shelburne JD, Crapo JO: Pulmonary morphology in a multihospital collaborative extracorporeal membrane oxygenation project. *Am J Pathol* 95:191-208, 1979.
- 93 Raymond RM, Harkema JM, Stoffs WV, Emerson TE: Effects of naloxone therapy on hemodynamics and metabolism following a superlethal dosage of *Escherichia coli* in dogs. *Surg Gynec Obstet* 152:159-162, 1980.

- 94 Rees M, Payne JG, Bowen JC: Naloxone reverses tissue effects of live *Escherichia coli* sepsis. *Surgery* 91:81-86, 1982.
- 95 Rinaldo JE, Rogers RM: Adult respiratory distress syndrome. Changing concepts of lung injury and repair. *New Eng J Med* 306:900-909, 1982.
- 96 Robin ED, Carey LC, Grenvik A, Glauser F, Gandio R: Capillary leak syndrome with pulmonary edema. *Arch Intern Med* 130:66-71, 1972.
- 97 Rådegran K, Bergentz S-E, Lewis DH, Lungqvist U, Olsson P: Pulmonary effects of induced platelet aggregation. Intramuscular obstruction or vasoconstriction? *Scand Lab Invest* 28:423-427, 1971.
- 98 Sachs T, Moldow CF, Craddock PR, Bowers TK, Jacob HS: Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. *J Clin Invest* 61:1161-1167, 1978.
- 99 Saldeen T: The microembolism syndrome, *Microvasc Res* 11:227-259, 1976.
- 100 Salerno TA, Milne B, Jhamadas KH: Hemodynamic effects of naloxone in hemorrhagic shock in pigs. *Surg Gyn Obstet* 152:773-776, 1981.
- 101 Samuelsson B, Goldyne M, Granström E, Hamberg M, Hammarström S, Malmsten C: Prostaglandins and thromboxanes. *Ann Rev Biochem* 47:997-1029, 1978.
- 102 Scheffel U, McIntyre PA, Evatt B, Dvornicky JA, Natarajan TK, Bolling DR, Murphy EA: Evaluation of indium-111 as a new high photon yield gammaemitting "physiological" platelet label. *The Johns Hopkins Medical Journal* 140:285-293, 1977.
- 103 Schmidt GB, O'Neill WW, Kotb K, Hwang KK, Bennet EJ, Bombeck CT: Continuous positive airway pressure in the prophylaxis of the adult respiratory distress syndrome. *Surg Gyn Obstet* 143:613-619, 1976.

- 104 Schneider RC, Zapol WM, Carvalho AC: Platelet consumption and sequestration in severe acute respiratory failure. *Am Rev Resp Dis* 122:445-451, 1980.
- 105 Schumer W: Steroids in the treatment of clinical septic shock. *Ann Surg* 184:333-341, 1976.
- 106 Sladen A: Methylprednisolone. Pharmacologic doses in shock lung syndrome. *J Thorac Cardiovasc Surg* 71:800-806, 1976.
- 107 Smith JB: Prostaglandins and platelet aggregation. *Acta Med Scand Suppl* 210:91-99, 1981.
- 108 Smith ME, Gunther R, Gee M, Flynn J, Demling RH: Leukocytes, platelets and thromboxane A₂ in endotoxin-induced lung injury. *Surgery* 90:102-107, 1981.
- 109 Spangulo PJ, Ellner JJ, Hassid A, Dunn MJ: Thromboxane A₂ mediate augmented polymorphonuclear leukocyte adhesiveness. *J Clin Invest* 66:406-414, 1980.
- 110 Staub NC: Pulmonary edema due to increased microvascular permeability. *Ann Rev Med* 32:291-312, 1981.
- 111 Staub NC, Schultz EL, Albertine KH: Leukocytes and pulmonary microvascular injury. *Ann NY Acad Sci* 384:332-343, 1982.
- 112 Stein M, Thomas DP: Role of platelets in the acute pulmonary responses to endotoxin. *J Appl Physiol* 23:47-52, 1967.
- 113 Stone CA, Wenger HC, Ludden CT, Stavorski JM, Ross CA: Antiserotonin-antihistamin properties of cyproheptadine. *J Pharmacol* 131:73-84, 1961.
- 114 The Pharmacologic basis of therapeutics. Sixth Edition. Goodman LS and Gilman A. pp 613-623.
- 115 Tiengo M: Naloxone in irreversible shock. *Lancet* p 690, 1980.
- 116 Thörne LJ, Kuenzig M, McDonald HM, Schwartz SI: Effect of denervation of a lung on pulmonary platelet trapping associated with traumatic shock. *Surgery* 88:208-214, 1980.

- 117 Vaage J, Nicolaysen G, Waaler BA: Aggregation of blood platelets and increased hydraulic conductivity of pulmonary exchange vessels. *Acta Physiol Scand* 98:175-184, 1976.
- 118 Vick JA, Mehlman B, Heiffer MH: Early histamin release and death due to endotoxin. *137:902-906*, 1971.
- 119 Walker L, Eiseman B: The changing pattern of posttraumatic respiratory distress syndrome. *Ann Surg* 181:693-697, 1975.
- 120 Weigel JA, Mitchell RA, Snyder III WH: Early positive end-expiratory pressure in the adult respiratory distress syndrome. *Arch Surg* 114:497-501, 1979.
- 121 White GL, Archer LT, Beller BK, Hinshaw LB: Increased survival with methylprednisolone treatment in canine endotoxin shock. *J Surg Res* 25:357-364, 1978.
- 122 Wilson JW: Treatment or prevention of pulmonary cellular damage with pharmacologic doses of corticosteroid. *Surg Gyn Obstet* 134:675-681, 1972.
- 123 Winslow EJ, Loeb HS, Rahimtoola SH, Kamath S, Gunnar RM: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. *Am J Med* 54:421-432, 1973.
- 124 Wong C, Flynn J, Demling RH: Role of oxygen radicals in endotoxin-induced lung injury. *Arch Surg* 119:77-82, 1984.
- 125 Yamazaki N, Okabe E, Hara A, Takahashi K, Ito H: Pharmacological study on endotoxin shock. 11th Eurp Conf Microcirculation. Basel, Krager S. 472-476, 1980.

PAPER I

EFFECT OF NALOXONE ON ENDOTOXIN-INDUCED PULMONARY PLATELET SEQUESTRATION

Per Almqvist, Marian Kuenzig, and Seymour I. Schwartz

*From the Department of Surgery, University of Rochester School of Medicine and Dentistry,
601 Elmwood Avenue, Rochester, New York 14642 (716) 275-2725*

(Submitted for publication February 15, 1982. Accepted August 25, 1982)

Abstract. The opiate antagonist, naloxone, has been shown to obviate hypotension and to improve survival of animals with shock induced by endotoxin, hypovolemia, and spinal transection. This study was done to evaluate the effects of naloxone on pulmonary platelet trapping (PPT) and on platelet aggregability in dogs with endotoxin shock. Dogs with ^{51}Cr labelled platelets were treated with naloxone before and after induction of endotoxin shock. PPT was assessed by measuring ^{51}Cr activity in lung and blood using a gamma scintillation counter. ADP induced platelet aggregability was measured in an aggregometer. PPT seen in endotoxin shocked controls was obviated by naloxone treatment; this effect was more pronounced in pretreated dogs. Naloxone treated animals did not show the increased platelet aggregability normally seen in endotoxin shocked dogs. These results suggest the applicability of naloxone therapy for adult respiratory distress associated with shock.

Pulmonary platelet trapping (PPT) is thought to play an important role in the evolution of the adult respiratory distress syndrome (ARDS). Injection of endotoxin is known to cause a decrease in circulating platelets and white blood cells, and an increase in platelet aggregability (Myrvold et al., 1977; Thorne et al., 1980); endotoxin is bound to the platelets and this complex is trapped in the lung (Bredenberg et al., 1980). White blood cell trapping has also been demonstrated in the lung (Myrvold & Svalander, 1977).

Endorphins are short polypeptides with opiate activity that have been identified in the brain and in the pituitary. The endorphin system is activated by stress (Rossier et al., 1977). In addition to their analgesic effects endorphins may also be implicated in the pathophysiology of shock (Faden & Holoday, 1980). Intravenous and intraventriculocisternal injection of β -endorphin produces profound hypotension and bradycardia. Naloxone, a specific opiate antagonist, administered either intrave-

nously or intraventriculocisternally effectively blocks these effects (Laubie et al., 1977). Naloxone is also effective in attenuating blood pressure drop and in increasing survival in hemorrhagic and in endotoxin shock (Faden & Holoday, 1980; Raymond et al., 1981; Curtis & Lefer, 1980).

This study was designed to evaluate the effect of naloxone on pulmonary platelet trapping, *in vitro* platelet aggregability, and hypotension in dogs subjected to endotoxin injection.

MATERIALS AND METHODS

Thirty-one mongrel dogs of both sexes weighing between 9–14 kg were used. One day prior to the shock study the dogs were anesthetized with pentobarbital sodium (30 mg/kg b.w.), endotracheally intubated, and ventilated by means of a Harvard respirator, 100 ml of blood were drawn from each dog, the platelets were tagged with ^{51}Cr according to a modification of a method described by Abrahamsen (Abrahamsen, 1968), and the autologous platelets were reinfused. On the following day the dogs were reanesthetized, reintubated, and ventilated. A catheter was inserted in the brachial artery and connected to a manometer. Shock was induced by an intravenous injection of 20 mg *E. coli* endotoxin (Difco) over a five-min period. The dogs were studied for two hours, then sacrificed.

Blood pressure was measured continuously during the shock period. Arterial blood samples were taken prior to induction of shock and also before sacrifice to determine ADP-induced platelet aggregability by a photoelectric method (Colytograph Damon/IEC Division, Needham Heights, Mass.), and ^{51}Cr activity in a gamma scintillation counter. At sacrifice, the lungs were excised, weighed, dried with pressurized air for four to five days, and reweighed. The pleura was peeled off the dried lungs to avoid counting blood on the surface, the lung parenchyma was divided and placed into tubes. ^{51}Cr activity of the blood and lung was counted in a gamma scintillation counter. The ratio of counts/g lung tissue to counts/g blood was determined and used as a measure of PPT. The wet/dry lung weight ratio was calculated and used as a measure of pulmonary edema.

Table I. Blood pressure drop (mmHg)

Group	N	5 min.	<i>p</i> ^a	90 min.	<i>p</i> ^a
I	5	0	—	10±8	—
II	6	0	—	0	—
III	5	61±29	—	71±26	—
IV	5	64±35	NS	62±37	NS
V	5	74±22	NS	48±8	<0.05
VI	5	47±20	NS	39±27	<0.05

^a In comparison to endotoxin controls.

Six groups were studied: group I, nonshocked controls sacrificed after four hours af anesthesia; group II, non-shocked dogs injected with naloxone 2 mg/kg b.w. i.v. at time 0 followed by 1 mg/kg b.w. at 30, 60, and 90 min; group III, endotoxin controls sacrificed after two hours of shock; group IV, low dose naloxone 2 mg/kg b.w. i.v. at 10 min. and 1 mg/kg b.w. at 30, 60, and 90 min postendotoxin; group V, high dose naloxone 10 mg/kg b.w. at ten, 30, 60, and 90 min after induction of endotoxin shock; and group VI, naloxone 2 mg/kg b.w. given before administration of endotoxin followed by 1 mg/kg b.w. at 30, 60, and 90 min after shock induction.

Statistical analysis was made with Student's *t*-test.

RESULTS

There was no change in blood pressure in the sham control group I, nor in the naloxone control group II. The mean blood pressure drop in the endotoxin control group III, recorded at five minutes after endotoxin injection, was 61 mmHg, and at 90 min after the endotoxin injection the mean drop was 71 mmHg. In group IV, which received low dose naloxone, the same blood pressure changes were recorded. By contrast, in group V receiving high dose naloxone postshock, although the initial blood pressure drop was the same, there was a significantly less sustained drop at 90 min. In the naloxone pretreated animals, group VI, there was a smaller blood pressure drop at five minutes which became significantly lower by 90 min after the injection of endotoxin (Table I).

Table II. Lung weight wet/dry ratio

Group	N	Mean ± SD
I	5	4.6±0.3
II	6	4.7±0.3
III	4	4.4±0.6
IV	4	4.3±1.2
V	5	4.8±0.3
VI	4	4.3±0.3

No significant difference.

Table III. ⁵¹Cr lung/blood ratio

Group	N	Mean value ± SD	<i>p</i> ^a
I	5	1.30±0.28	<0.005
II	6	1.45±0.43	<0.005
III	5	3.76±1.43	—
IV	5	2.50±1.28	<0.10
V	5	2.64±1.40	<0.15
VI	5	2.00±0.35	<0.025

^a In comparison to endotoxin controls.

There was no significant difference in the wet/dry lung weight ratios among any of the groups (Table II).

There was no significant difference in the ⁵¹Cr lung/blood ratio between the sham control group I and the naloxone control group II. The ⁵¹Cr lung to blood ratio in the endotoxin control group III was significantly higher than in both groups I and II (*p*<0.005). Both the naloxone postshock treated groups IV and V had ⁵¹Cr lung/blood ratios that were lower than the untreated endotoxin shocked dogs, but still above the control values. Naloxone administered prior to endotoxin, group VI, had a more marked effect (Table III).

There was no change in platelet aggregability in the sham control group I nor in the naloxone control group II. In the endotoxin control group III an increased aggregability was noted in most dogs; in an occasional animal there was decreased ADP-induced aggregability because aggregation had occurred prior to the addition of ADP. In all naloxone treated groups, IV, V and VI, platelet aggregability was either unchanged or decreased (Table IV).

DISCUSSION

Adult respiratory distress syndrome (ARDS) is a serious consequence of shock induced by different causes and is accompanied by a high mortality

Table IV. ADP-induced platelet aggregability

Group	No change (Dogs)	Increased (Dogs)	Decreased (Dogs)
I	5		
II	6		
III		4	1
IV	3		2
V	2		3
VI	2		3

(Landesman & Gorbach, 1978). The evolution of ARDS is complex and is as yet not fully understood. An early manifestation of endotoxin, hemorrhagic, and traumatic shock is a sharp fall in circulating platelets accompanied by increased platelet aggregability. This decrease in the circulating platelets is accompanied by sequestration of platelets in the lung (Ljungqvist et al., 1971; Ljungqvist & Schwartz, 1975; Ljungqvist, 1973). In addition to the physical factor of platelet plugs, the release of platelet vasoactive substances from the aggregating platelet may contribute to pulmonary vasoconstriction (Ljungqvist & Schwartz, 1975). This in turn is followed by an increase in pulmonary artery pressure and a decrease in cardiac output (Myrvold et al., 1977). The platelets are known to contain serotonin which is released when platelets are trapped in the lung. The increase in pulmonary artery pressure is not seen in endotoxin shocked dogs previously made thrombocytopenic or treated with antiserotonin drugs (Myrvold et al., 1977; Stein & Thomas, 1967).

It has also been shown that the complement system is activated by endotoxin injection and by traumatic shock (Heideman et al., 1979). In response to complement activation white blood cells aggregate, become trapped in the lung and contribute to endothelial damage (Jacob et al., 1980). Intravenous injection of fragment D, a purified fibrinogen degradation product, causes decrease in circulating platelets, pulmonary dysfunction, vascular permeability to albumin, neutrophil congestion, and complement activation in rabbits. This response does not occur in thrombocytopenic rabbits (Manwaring & Curreri, 1980). These studies suggest a role of pulmonary platelet trapping early in the evolution of ARDS. The detrimental effects of PPT occurs very early in endotoxin shock and there is a significant return of circulating platelets at three hours. We chose two hours as our observation endpoint because substantial PPT is still present at this time in endotoxin shocked dogs (Heideman et al., 1979). Pulmonary edema, however, is a later development and we were unable to show an increase in the wet/dry lung weight ratio at two hours. We did not use the more sensitive green-dye double indicator dilution method to measure extravascular lung water because it might have interfered with our technique for PPT measurement, the primary purpose of this study.

Stressful stimuli have been shown to increase

the amount of circulating β -endorphin and of ACTH. These polypeptides appear to be concomitantly released from the anterior lobe of the pituitary gland into the systemic circulation (Guillemin et al., 1977). β -endorphin is thought to gain entrance to the central nervous system, and through stimulation of opiate receptors in the CNS, to produce hypotension and bradycardia (Faden & Holoday, 1980). All these effects can be blocked by injecting naloxone, a specific opiate antagonist. Naloxone has also been shown to be effective in restoring blood pressure and in increasing survival in dogs subjected to hemorrhagic or to endotoxin shock (Faden & Holoday, 1980; Raymond et al., 1981). Furthermore, naloxone decreases the concentration of proteolytic enzymes and myocardial depressant factors in hemorrhagic shocked cats; attenuates the increase in hematocrit, and the severe acidosis and hypoglycemia in endotoxin shocked dogs; and prevents the drop in WBCs in endotoxin shocked mice (Curtis & Lefer, 1980; Raymond et al., 1981; Wright & Weller, 1980).

Our work has shown that naloxone is effective in reducing pulmonary platelet trapping, attenuating the profound blood pressure drop, and preventing the increased platelet aggregability in endotoxin shocked dogs. This, together with the previously cited positive effects of naloxone, and several case reports showing naloxone to be effective in humans with septic shock (Peters et al., 1981), strengthens the indication for an opiate antagonist as an adjunct in the treatment of septic shock.

ACKNOWLEDGEMENT

Supported by N. I. H. Grant No. HL 13466. Naloxone HCl kindly supplied by Endo Laboratories, Garden City, New York.

REFERENCES

- Abrahamsen, A. F. 1968. A modification of the technique for ^{51}Cr -labelling of blood platelets giving increased circulating platelet activity. *Scand J Haematol* 5, 53-63.
- Bredenberg, C. E., Taylor, G. A. & Webb, W. R. 1980. The effect of thrombocytopenia on the pulmonary and systemic hemodynamics of canine endotoxin shock. *Surgery* 87, 59-68.
- Curtis, M. T. & Lefer, A. M. 1980. Protective actions of naloxone in hemorrhagic shock. *Am J Physiol* 239, H416-H421.

- Faden, A. J. & Holoday, J. W. 1980. Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. *J Infect Dis* 142, 229-238.
- Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. & Bloom, F. 1977. β -endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science*, Sept; 1367-1369.
- Heideman, M., Kaijser, B. & Gelin, L. E. 1979. Complement activation early in endotoxin shock. *J Surg Res* 26, 74-78.
- Jacob, H. S., Craddock, P. R., Hammerschmidt, D. E. & Moldow, C. F. 1980. Complement-induced granulocyte aggregation. *New Engl J Med* 302, 789-794.
- Landesman, S. H. & Gorbach, S. L. 1978. Gram negative sepsis and shock. *Orthop Clin North Am* 9, 611-625.
- Laubie, M., Schmitt, H., Vincent, M. & Remond, G. 1977. Central cardiovascular effects of morphinomimetic peptides in dog. *Eur J Pharmacol* 46, 67-71.
- Ljungqvist, U. 1978. Platelets in shock and trauma. *J Surg Res* 15, 132-162.
- Ljungqvist, U., Bergentz, S. E. & Lewis, D. H. 1971. The distribution of platelets, fibrin and erythrocytes in various organs following experimental trauma. *Eur Surg Res* 3, 293.
- Ljungqvist, U. & Schwartz, S. I. 1975. Pulmonary platelet trapping during shock and pulmonary embolism. *J Surg Res* 18, 559-565.
- Manwaring, D. & Curreri, P. W. 1980. Platelet: mediation of fragment D-induced respiratory distress syndrome. *Surg Forum* 31, 242-243.
- Myrvold, H. D., Brandberg, A., Lindqvist, O., Busch, C. & Lewis, D. H. 1977. The role of platelets and fibrinogen in experimental septic shock. *Acta Chir Scand* 143, 131-137.
- Peters, W. P., Johnson, M. W., Friedman, P. A. & Mitch, W. E. 1981. Pressor effect of naloxone in septic shock. *Lancet*, March, 529-532.
- Raymond, R. M., Harkema, J. M., Stoff, W. V., and Emerson, T. E. Jr. 1981. Effects of naloxone therapy on hemodynamics and metabolism following a superlethal dosage of Escherichia Coli endotoxin in Dogs. *Surg Gynecol Obstet* 152, 159-162.
- Rossier, J., French, E. D., Rivier, C., Ling, N., Guillemin, R. & Bloom, F. E. 1977. Foot-shock induced stress increases β -endorphin levels in blood but not brain. *Nature* 270, 618-620.
- Stein, M. & Thomas, D. P. 1967. Role of platelets in the acute pulmonary response to endotoxin. *J Appl Physiol* 23, 47-52.
- Thörne, L. J., Kuenzig, M., McDonald, H. M. & Schwartz, S. I. 1980. Effect of denervation of a lung on pulmonary platelet trapping associated with traumatic shock. *Surgery* 88, 208-214.
- Wright, D. I. M. & Weller, M. P. I. 1980. Neuropharmacological agents modifying endotoxin-induced changes in mice. *J R Soc Med* 73, 431-436.

PAPER II

The Effect of Naloxone and Cyproheptadine on Pulmonary Platelet Trapping, Hypotension, and Platelet Aggregability in Traumatized Dogs

P. ALMQVIST, M.D., M. KUENZIG, B.S., AND S. I. SCHWARTZ, M.D., F.A.C.S.

Adult respiratory distress syndrome (ARDS) is a serious complication of trauma and sepsis. We have earlier shown naloxone, an opiate antagonist, and cyproheptadine, an antiserotonin drug, to be effective in reducing pulmonary platelet trapping (PPT), which is thought to play an important role in the evolution of ARDS in endotoxin-shocked dogs. Endorphins are implicated as pathophysiologic factors in shock, and serotonin is a possible mediator of their action. The present study shows naloxone and cyproheptadine to be equally effective in protecting against PPT in dogs subjected to trauma, and when naloxone is given before the trauma it also obviates the hypotension associated with trauma. In addition, the naloxone- and cyproheptadine-treated animals did not show the increased platelet aggregability usually seen in traumatized dogs.

The opiocortin system is activated by various kinds of stress. Increased amounts of circulating β -endorphin have been determined in rats subjected to endotoxin injection, to electric foot shock and to unilateral tibia-fibula fracture (9, 18, 20). Recent literature has implicated β -endorphin as a pathophysiologic factor in shock (8). Injection of β -endorphin causes hypotension and bradycardia; these effects can effectively be blocked by naloxone, an opiate antagonist, and by antiserotonin drugs, the latter suggesting a serotonergic pathway for β -endorphin activity (11, 12).

Soft-tissue trauma is associated with increased platelet aggregability and sequestration of platelets in the lung (4, 15). Pulmonary platelet trapping (PTT) is thought to play an important role in the pathogenesis of adult respiratory distress syndrome (ARDS) (14), a major complication of trauma and sepsis associated with a high mortality rate.

We have previously shown both naloxone and cyproheptadine, an antiserotonin drug, to be effective in reducing PPT associated with endotoxin shock (2; unpublished data). The present study was done to evaluate the effects of naloxone and of cyproheptadine on PPT, platelet aggregability, and hypotension in dogs subjected to hind-leg trauma.

MATERIALS AND METHODS

Thirty-seven adult mongrel dogs of either sex weighing 9 to 14 kg were used. Twenty-four hours before trauma the dogs were anesthetized with pentobarbital sodium (30 mg/kg B.W.), endotracheally intubated, and ventilated with room air by means of a Harvard respirator. Blood (100 ml) was drawn from each dog, the platelets were tagged with ^{51}Cr according to a modification of a method described by Abrahamsen (1), and the autologous platelets were reinfused.

On the following day the dogs were reanesthetized, reintubated, and ventilated. A catheter was inserted in the right brachial artery and connected to a manometer. Trauma was induced by 200 blows with a padded mallet to each hind limb, according to Blalock's protocol (5). The dogs were studied for 4 hours, then sacrificed. Blood pressure was measured continuously during the study. Arterial blood samples were taken before the trauma and also before sacrifice to determine ADP-induced platelet aggregability by a photoelectric method (Colysagrapb Damon, Division IEC, Needham Heights, MA) and for measurement of ^{51}Cr activity.

At sacrifice, the lungs were excised, weighed, dried with pressurized air for 4 to 5 days, and reweighed. The pleura was peeled off the dried lungs to avoid counting blood on the surface, and the lung parenchyma divided and placed into tubes. ^{51}Cr activity of the blood and lung samples was counted in a gamma scintillation counter. The ratio of counts/gm lung tissue to counts/gm blood was determined and used as a measure of PPT. The wet/

From the Department of Surgery, University of Rochester School of Medicine and Dentistry Medical Center, Strong Memorial Hospital, Rochester, New York. Supported by NIH Grant HL 13466.

Address for reprints: Per Almqvist, M.D., Department of Surgery, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642.

dry lung weight ratio was calculated and used as a measure of pulmonary edema.

Seven groups were studied: Group I, nontraumatized controls sacrificed after 4 hours of anesthesia; Group II, nontraumatized dogs injected with naloxone 2 mg/kg B.W. IV at time 0 followed by 1 mg/kg B.W. IV every 30 minutes; Group III, nontraumatized dogs injected with cyproheptadine 1 mg/kg B.W. IV at 0, 30, and 60 minutes; Group IV, trauma controls sacrificed after 4 hours; Group V, traumatized dogs treated with naloxone 2 mg/kg B.W. IV 30 minutes post-trauma and 1 mg/kg B.W. IV every 30 minutes; Group VI, traumatized dogs treated with naloxone 2 mg/kg B.W. IV 10 minutes before trauma followed by 1 mg/kg B.W. IV every 30 minutes post-trauma; Group VII, traumatized dogs treated with cyproheptadine 1 mg/kg B.W. IV 30 and 60 minutes post-trauma. Statistical analysis was made with the two-tailed Student's *t*-test. A value of *p* less than 0.05 was considered significant.

RESULTS

There were no blood pressure changes in Groups I, II, and III. In Group IV, the untreated traumatized dogs, the blood pressure fell 21 ± 14 mm Hg during the first 15 minutes and returned to control values within 30 minutes. The same blood pressure changes were observed in Groups V and VII treated post-trauma with naloxone or cyproheptadine, respectively. In Group VI, treated with naloxone before trauma, the blood pressure drop was significantly less (2 ± 4.5 mm Hg; $p < 0.01$) (Table I).

The ADP-induced platelet aggregability was unchanged in the control Groups I, II, and III. In the trauma controls (Group IV) there was a small but definite increase. This increase was not seen in any of the treated dogs (Groups V, VI, and VII).

There was no significant difference in the wet/dry lung weight ratios, probably because the lung edema takes longer than 4 hours to develop (Table II). There was no significant difference in the ^{51}Cr lung/blood ratios among the control Groups I, II, and III. The trauma control

TABLE I
Blood pressure

Group	n	Drop in mm Hg (mean $\pm$ SD)	<i>P</i> value*
I	5	0	—
II	6	0	—
III	5	0	—
IV	6	21 ± 14	—
V	5	22 ± 9	NS
VI	5	2 ± 4.5	<0.01
VII	5	25 ± 6	NS

NS = Not significant.

* = in comparison to trauma control (Group IV).

TABLE II
Wet/dry lung weight ratio

Group	n	Mean $\pm$ SD	<i>P</i> value*
I	5	4.6 ± 0.3	—
II	6	4.7 ± 0.3	NS
III	4	4.3 ± 0.3	NS
IV	4	4.8 ± 0.5	NS
V	4	4.8 ± 0.2	NS
VI	4	4.6 ± 0.3	NS
VII	5	4.3 ± 0.3	NS

NS = Not significant.

* = in comparison to sham (Group I).

TABLE III
 ^{51}Cr lung/blood ratios

Group	n	Mean $\pm$ SD
I	5	1.30 ± 0.28
II	6	1.45 ± 0.43
III	5	1.38 ± 0.37
IV	6	1.73 ± 0.34
V	5	1.43 ± 0.14
VI	5	1.32 ± 0.18
VII	5	1.25 ± 0.12

TABLE IV
 ^{51}Cr lung/blood ratio comparisons of group mean values

Groups compared	<i>P</i> value
Sham vs. naloxone control	NS
Sham vs. cyproheptadine control	NS
Sham vs. trauma control	<0.025
Naloxone post-treated vs. trauma control	<0.05
Naloxone post-treated vs. naloxone control or sham	NS
Naloxone pretreated vs. trauma control	<0.025
Naloxone pretreated vs. naloxone control or sham	NS
Cyproheptadine post-treated vs. trauma control	<0.01
Cyproheptadine post-treated vs. cyproheptadine control or sham	NS

NS = Not significant.

Group IV had a significantly higher ^{51}Cr lung/blood ratio than the sham control Group I ($p < 0.025$). Treatment with cyproheptadine after trauma, and both pretreatment and post-treatment with naloxone, resulted in ^{51}Cr lung/blood ratios significantly lower than those of the trauma controls, and not significantly different from shams (Tables III & IV).

DISCUSSION

Trauma is the leading cause of morbidity and mortality in Americans under the age of 45 years and the third

highest overall cause of death (3). ARDS is a serious complication of major trauma, sepsis, and burns. Trauma alone has been shown to cause ARDS, and the addition of sepsis, which is often the case, makes the lung injury worse (19). The etiology of ARDS is not fully understood, but sequestration of platelets and leukocytes which form microthrombi in pulmonary vessels, release of vasoactive substances such as serotonin, histamine, and thromboxane A_2 from platelets, and activation of the complement system have been implicated as mediators of lung injury.

Studies using either injection of β -endorphin or blocking of opiate receptors in various shock models provide ample evidence that the opiocortin system is involved in the pathophysiology of shock. In hemorrhagic shock in dogs (10), cats (6), and rats (7), naloxone significantly improved arterial pressure, cardiovascular parameters, and rate of survival. Naloxone is also effective in attenuating hypotension and improving survival rates in rats (8) and dogs (17) subjected to endotoxin injection. Our own work has shown naloxone to be effective in reducing PPT, hypotension, and platelet aggregability in endotoxin-shocked dogs (2). β -endorphin injected either intraventricularly or intravenously causes hypotension and bradycardia. These effects can be blocked by naloxone and by antiserotonin drugs (11, 12). We have also shown that cyproheptadine, a very potent antiserotonin drug, blocked PPT when given after induction of endotoxin shock but had no effect when administered before endotoxin injection (unpublished data). For this reason we did not include a group pretreated with cyproheptadine in this study.

Activation of the opiocortin system and release of β -endorphin due to trauma in dogs has not been reported. The present study shows naloxone to be effective in obviating PPT and when given before trauma it also abolishes the hypotension. This is indirect evidence that the opiocortin system may be activated and β -endorphin released by trauma and also that the endogenous opiates are responsible for the PPT and hypotension in this trauma model.

The treatment of ARDS associated with traumatic shock, in addition to ventilatory assistance with positive end expiratory pressure, includes pharmacologic doses of steroids. It has been shown that β -endorphin and ACTH are concomitantly released from the anterior pituitary in stressed rats, and it has been postulated that some of the salutary effects of steroids may be due to a negative feedback which shuts off both ACTH and β -endorphin release (16). However, the use of steroids as treatment for ARDS has recently been challenged and Lucas and

Ledgerwood's work questions the benefit of steroid therapy (13).

Naloxone has been shown to be effective in increasing blood pressure in patients in shock refractory to usual shock therapy (16). Our work strengthens the indication for naloxone as a tool in the treatment of ARDS associated with trauma and sepsis. Cyproheptadine, given after the trauma, is equally effective in reducing PPT.

REFERENCES

1. Abrahamson, A. F.: A modification of the technique for ^{14}C labeling of blood platelets giving increased platelet activity. *Scand. J. Haematol.*, 5: 53-63, 1968.
2. Almqvist, P., Kuening, M., Schwartz, S. I.: Effect of naloxone on endotoxin induced pulmonary platelet sequestration. *Surg. Forum.*, 32: 304-306, 1981.
3. Baker, C. C., Oppenheimer, L., Stephens, B., et al.: Epidemiology of trauma deaths. *Am. J. Surg.*, 140: 144-148, 1980.
4. Bergentz, S. E., Lewis, D. H., Lyngqvist, U.: Trapping of platelets in the lung after experimental injury. *Sixth European Conference on Microcirculation*. Basel, S. Karger, 1971, pp. 35-40.
5. Blalock, A.: Experimental shock: The cause of the low blood pressure produced by muscle injury. *Arch. Surg.*, 30: 959-986, 1930.
6. Curtis, M. T., Lefer, A. M.: Protective actions of naloxone in hemorrhagic shock. *Am. J. Physiol.*, 239: 416-421, 1980.
7. Faden, A. J., Holoday, J. W.: Opiate antagonists: A role in the treatment of hypovolemic shock. *Science*, 206: 317-318, 1979.
8. Faden, A. J., Holoday, J. W.: Experimental endotoxin shock: The pathophysiological function of endorphins and treatment with opiate antagonists. *J. Infect. Dis.*, 2: 229-238, 1980.
9. Guillemin, R., Vargo, T., Rossier, J., et al.: β -endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science*, 197: 1367-1369, 1977.
10. Gurli, N. J., Vargish, T., Reynolds, D. G., et al.: Opiate receptors and endorphins in the pathophysiology of hemorrhagic shock. *Surgery*, 89: 364-369, 1981.
11. Laubie, M., Schmitt, H., Vincent, M., et al.: Central cardiovascular effects of morphinomimetic peptides in dogs. *Eur. J. Pharmacol.*, 46: 67-71, 1977.
12. Lemaire, J., Tseng, R., Lemaire, S.: Systemic administration of β -endorphin: Potent hypotensive effect involving a serotonergic pathway. *Proc. Nat. Acad. Sci. U.S.A.*, 75: 6240-6242, 1978.
13. Lucas, C. E., Ledgerwood, A. M.: Pulmonary response of massive steroids in seriously injured patients. *Ann. Surg.*, 194: 256-260, 1981.
14. Myrvold, H. D., Brandberg, A., Lindqvist, U., et al.: The role of platelets and fibrinogen in experimental septic shock. *Acta Chir. Scand.*, 143: 131-137, 1977.
15. Peer, R. M., Schwartz, S. I.: Prevention of pulmonary platelet trapping following trauma. *Surg. Forum.*, 24: 5-7, 1973.
16. Peters, W. P., Johnson, M. W., Friedman, P. A., et al.: Pressor effect of naloxone in septic shock. *Lancet*, i: 529-532, 1981.
17. Raymond, R. M., Harkema, J. M., Stoffa, N. V., et al.: Effects of naloxone therapy on hemodynamics and metabolism following a sup lethal dosage of *Escherichia coli* endotoxin in dogs. *Surg. Gynecol. Obstet.*, 152: 159-162, 1981.
18. Rossier, J., French, E. D., Rivier, C., et al.: Post-shock induced stress increases β -endorphin levels in blood but not brain. *Nature*, 270: 618-620, 1977.
19. Walker, L., Eisenman, B.: The changing patterns of post-traumatic respiratory distress syndrome. *Ann. Surg.*, 181: 693-697, 1975.
20. Yamazaki, N., Okabe, E., Hara, A., et al.: Pharmacological study on endotoxin shock. *Eleventh European Conference on Microcirculation*. Basel, S. Karger, 1980, pp. 472-476.

PAPER III

TITLE: Treatment of Experimental Canine Endotoxin Shock with Ibuprofen, a Cyclooxygenase Inhibitor

AUTHORS: Per M. Almqvist, Marian Kuenzig and Seymour I Schwartz

AFFILIATION: Department of Surgery, The University of Rochester School of Medicine and Dentistry, Medical Center, Strong Memorial Hospital, 601 Elmwood Avenue, Rochester, New York 14642

Correspondence: Per M. Almqvist, M.D.
Department of Surgery
Strong Memorial Hospital
601 Elmwood Avenue
Rochester, New York 14642

Running Head: Ibuprofen in endotoxin shock

ABSTRACT

The arachidonic acid derivate thromboxane A₂, a very potent platelet aggregator, is increased in endotoxin shock. Ibuprofen blocks the formation of thromboxane A₂ and has antiplatelet and antileukocyte aggregability properties.

The effects of ibuprofen on pulmonary platelet trapping, platelet and leukocyte counts, platelet aggregability, hematocrit, and blood pressure were evaluated in endotoxin shocked dogs. The initial drop in blood pressure and in leukocyte and platelet counts seen in endotoxin shock was not altered by ibuprofen treatment. At two hours the ibuprofen treated dogs had less hypotension compared to endotoxin control. Platelet counts were also higher in the ibuprofen treated dogs at two hours. Significant recovery of leukocytes was seen only when pretreatment was used. Pulmonary platelet trapping was significantly lower in the ibuprofen treated dogs compared to endotoxin controls and not significantly different from the sham dogs when ibuprofen was given before endotoxin injection.

This study demonstrates the efficacy of ibuprofen not only in reducing pulmonary platelet trapping but also in obviating the late hypotension in experimental endotoxin shock.

Key words: ARDS, endotoxin shock, hypotension, pulmonary platelet trapping, ibuprofen

INTRODUCTION

The adult respiratory distress syndrome (ARDS) is a serious complication of trauma and sepsis. The etiology is as yet not fully understood, but sequestration of platelets (1, 2, 3), white blood cells (4), release of vasoactive substances such as serotonin (5), histamine (6), catecholamines (7) and bradykinin (8) are thought to be important factors.

Recently it has been shown that early in endotoxin shock there is an increase in the arachidonic acid derivate thromboxane A_2 (TxA_2) (9). This substance is a very potent platelet aggregator, inducer of the platelet release reaction and constrictor of smooth muscles (10). These effects act in opposition to another arachidonic acid derivate, prostacyclin (PGI_2) (11), which, under normal conditions, seems to balance the effects of thromboxane A_2 (12). Under conditions of stress, such as shock, thromboxane A_2 production is increased (12). Ibuprofen is a cyclooxygenase inhibitor blocking the synthesis of both TxA_2 , and PGI_2 and has been shown to improve blood pressure, cardiac index and arterial pH (13) and to prevent platelet aggregation in canine myocardial ischemia (14).

This study was designed to show the effects of ibuprofen on blood pressure, pulmonary platelet trapping (PPT), ADP-induced platelet aggregability, peripheral platelet and white blood cell counts, hematocrit and wet/dry lung weight in an LD_{100} canine endotoxin shock model.

MATERIAL AND METHODS

Thirty-two adult mongrel dogs of both sexes weighing between 9-14 kg were used. One day prior to the shock study the dogs were anesthetized with pentobarbital sodium (30 mg/kg b.w.), endotracheally intubated, and ventilated by means of a Harvard respirator with room air. 100 ml of whole blood was drawn from each dog, the platelets were tagged with ^{51}Cr according to a modification of a method described by Abrahamsen (15) and the autologous platelets were reinfused. On the following day the dogs were reanesthetized, reintubated and ventilated. A catheter was positioned in the femoral artery and connected to a pressure gauge. Shock was induced by an intravenous injection of 2 mg/kg E. Coli endotoxin, Lipopolysaccharide W, E. Coli 0128:B12 Difco Laboratories, Detroit, Michigan, in

10 ml normal saline over a five minute period. The dogs were studied for two hours and then sacrificed. Blood pressure was monitored continuously during the experiment. Arterial blood samples were drawn prior to the induction of shock and at 10, 30, 60 and 120 minutes postshock. Hematocrit levels were determined in duplicate in microhematocrit tubes. Platelets and white blood cells were counted in a counting chamber under light microscopy. The ADP-induced platelet aggregability was determined by a photoelectric method, Colysagraph, Damon/IEC Division, Needham Heights, Massachusetts. At sacrifice, the lungs were excised, weighed and dried with pressurized air for four to five days and reweighed. The pleura was peeled off the dried lungs to avoid counting blood on the surface; the lung parenchyma was divided and placed into tubes. ^{51}Cr activity of the blood and lung was counted in a gamma scintillation counter. The ratio of counts/g lung tissue to counts/g PPT was determined as a measure of pulmonary platelet trapping (PPT). The wet/dry lung weight ratio was calculated as a measure of pulmonary edema.

Five groups were studied: Group I, nonshocked controls sacrificed after four hours of anesthesia; Group II, nonshocked dogs injected with ibuprofen 10 mg/kg b.w. i.v. at 0 and 60 minutes; Group III, endotoxin control, sacrificed after two hours of shock; Group IV, ibuprofen 10 mg/kg b.w. i.v. at 10 and 60 minutes after the injection of endotoxin; and Group V, ibuprofen 10 mg/kg b.w. i.v. 10 minutes before endotoxin injection and 60 minutes after the induction of shock. Two separate groups of dogs were used for Hct, peripheral platelet and WBC counts on the sham and endotoxin controls. Statistical significance of difference was calculated using Wilcoxon's rank sum test. A p-value of less than 0.05 was considered significant.

RESULTS

The blood pressure was stable during the observation period in sham control Group I and in the ibuprofen control Group II. The mean blood pressure drop in the endotoxin control Group III, recorded at five minutes after endotoxin injection was 61 mm Hg/mm Hg and at 120 minutes after endotoxin injection the mean drop from control levels was 74 mm Hg. In the endotoxin dogs treated with ibuprofen the same initial drops were seen, but the blood pressure drop at 120 minutes postendotoxin injection was less and not significantly different from sham (Table I).

There was no significant difference in the wet/dry lung weight ratios between any of the groups.

There was no significant difference in the ^{51}Cr lung/blood ratios between the sham controls in Group I and the ibuprofen controls in Group II. The ^{51}Cr lung/blood ratio in the endotoxin control, Group III, was significantly higher than in Groups I and II. Group IV, which was treated with ibuprofen after the induction of shock had a significantly lower ^{51}Cr lung/blood ratio than the endotoxin control Group III but it was still significantly higher than the sham control, Group I. Group V, treated with ibuprofen before as well as after endotoxin injection, had a ^{51}Cr lung/blood ratio significantly lower than endotoxin control and not significantly different from the sham control (Table II).

The ADP-induced platelet aggregability was unchanged in Groups I and II. Some endotoxin control dogs showed an increased aggregability, others showed aggregation before ADP was added, which indicates a large increase in platelet aggregability. The ibuprofen treated dogs had platelets with aggregability slightly decreased from control values.

There was no increase in the hematocrit in Groups I and II. In Groups III, IV and V there was a significant increase compared to sham controls and there was no significant difference between these groups (Table II).

The platelet counts in Groups I and II were stable during the observation period. In all the endotoxin injected animals there was a significant drop in platelet counts at ten minutes, at 120 minutes, however, only the untreated endotoxin injected animals had a significant drop compared to sham control, Group I (Table II).

The WBC counts in Group I and II were stable during the study. The initial drop in WBC counts recorded at 10 minutes in Groups III, IV and V was significantly different compared to control Group I. The drop in peripheral WBC recorded at 120 minutes in endotoxin control and ibuprofen posttreated animals was still significantly different from control Group I, but the drop was less in Group IV. The WBC loss in Group V, ibuprofen pretreated, was significantly less compared to Group III, endotoxin control and not significantly different compared to sham control, Group I, at 120 minutes. (Table II).

DISCUSSION

Sepsis is the leading cause of the adult respiratory distress syndrome. The etiology is still obscure and under considerable debate. The endotoxin induced lung injury is a thoroughly studied animal model and is considered to be similar to that seen in clinical sepsis (16).

The evolution of ARDS in animal models can be divided into two phases. Phase I, is characterized by an increase in pulmonary artery pressure, sequestration of platelets and leukocytes in the pulmonary vessels, release of vasoactive substances such as serotonin and prostaglandins from these cells, and activation of the complement system (16, 17, 18). It is probably during this phase that the injury to the capillary endothelium is initiated (4). Jacob, et al. have demonstrated that complement-activated white blood cells are capable of destroying endothelial cells in vitro and that this capability is significantly augmented when platelets are present, or when serotonin is added (19). Phase II is characterized by an increase in permeability of pulmonary vessels to protein, indicative of endothelial damage (16).

The present model is designed to show the trapping of platelets in the lung, an early manifestation of shock that happens before significant pulmonary edema has developed. This explains the lack of any difference in the wet/dry lung weight ratios.

During endotoxin shock a number of arachidonic acid derivatives are produced (18, 20). Among these, two are of special interest because of their ability to modulate platelet and leukocyte behavior (21). TxA_2 is a very potent platelet aggregator and vasoconstrictor (10). Many cells including platelets, pulmonary parenchymal cells and leukocytes are capable of synthesizing TxA_2 (10). PGI_2 , besides being one of the most potent inhibitors of platelet aggregation, also relaxes vascular smooth muscles, stabilizes membranes and inhibits TxA_2 formation (11,22). Under normal conditions these two substances seem to balance each other. In the first phase of endotoxin shock there is an increase of TxA_2 and a relatively smaller increase of PGI_2 (12). When PGI_2 is injected into endotoxin shocked sheep the pulmonary artery hypertensive phase is attenuated and the protein leakage of Phase II is less than that seen in the untreated model (23). These same positive effects are seen when TxA_2 formation is

blocked by imidazole, a TxA_2 synthetase inhibitor (24). Indomethacin, a cyclooxygenase inhibitor, lessens the increase in pulmonary artery pressure; however it increases the protein leakage of Phase II (25). Indomethacin and many other nonsteroidal agents including ibuprofen, block the formation of both TxA_2 and PGI_2 while imidazole blocks only the formation of TxA_2 (10,13). Ibuprofen does not increase the protein leakage of Phase II and seem to have other positive effects compared to indomethacin. Ibuprofen has been shown to significantly improve arterial blood pressure, cardiac index and arterial pH in dogs subjected to endotoxin shock (13). Furthermore Jacob has demonstrated that ibuprofen is a strong inhibitor of granulocyte aggregation (19). The mechanism of these effects is not known.

The present study confirms the positive effects of ibuprofen on hypotension in endotoxin shock. The fact that we have used a lower dosage of ibuprofen than other investigators is offered as explanation for the blood pressure increase to less than preshock values. We chose to use this lower dose since a dosage of 25 mg/kg b.w. used in a pilot study, although it increased blood pressure to above control values, also increased PPT, the opposite of the desired effect. We have demonstrated that ibuprofen treatment at a moderate dosage significantly decreases PPT compared to endotoxin control; when it is given before endotoxin injection there is no significant difference in PPT compared to nonshocked controls. This prevention of PPT is accompanied by a return of circulating platelets and white blood cells, which we interpret as a release of platelets and WBC temporarily sequestered in the lung despite the ibuprofen injection. Pretreatment with ibuprofen did not have any effect on the initial hypotension and decrease in circulating platelets and WBC which is apparently not thromboxane dependent. We believe that the positive results from this experiment indicate that ibuprofen merits further study as an adjunct in the treatment of septic shock. Investigations using ibuprofen in combination with other drugs proven to be effective in increasing cardiac performance and lessening the pulmonary injury of shock are now in progress.

ACKNOWLEDGEMENTS

This study was supported by N.I.H. Grant No. HL 13466.
Ibuprofen was kindly supplied by the Upjohn Company,
Kalamazoo, Michigan.

REFERENCES

- 1 Schneider RC, Zapol WM, Carvalho AC: Platelet consumption and sequestration in severe acute respiratory failure. *Am Rev Respir Dis* 122:445-51, 1980.
- 2 Stein M, Thomas DP: Role of platelets in the acute pulmonary responses to endotoxin. *J Appl Physiol* 23:47-52, 1967.
- 3 Vaage I, Nicolaysen G, Waaler BA: Aggregation of blood platelets and increased hydraulic conductivity of pulmonary exchange vessels. *Acta Physiol Scand* 98:175-184, 1976.
- 4 Myrvold HE, Svalander C: Pulmonary microembolism in early experimental septic shock. *J Surg Res* 23:65-74, 1977.
- 5 Kusajima K, Ozdemir IA, Webb WR, Wax SD, Parker FB: Role of serotonin and serotonin antagonists on pulmonary hemodynamics and microcirculation in hemorrhagic shock. *J Thoracic Cardiovasc Surg* 67:908-914, 1974.
- 6 Vick JA, Mehlman B, Heiffer H: Early histamine release and death due to endotoxin. *Proc Soc Exp Biol Med* 137:902-906, 1971.
- 7 Hall RC, Hodge RL: Vasoactive hormones in endotoxin shock: A comparative study in cats and dogs. *J Physiol* 213:69-84, 1971.
- 8 Pang LM, O'Brodovich HM, Mellins RB, Stalcup SA: Bradykinin-induced increase in pulmonary vascular permeability in hypoxic sheep. *J Appl Physiol* 52:370-375, 1982.
- 9 Cook JA, Wise WC, Halushka PV: Elevated thromboxane levels in the rat during endotoxin shock. *J Clin Invest* 65:227-230, 1980.
- 10 Samuelsson B, Goldyne M, Granström E, Hamberg M, Hammarström S, Malmsten C: Prostaglandins and thromboxanes. *Ann Rev Biochem* 47:997-1029, 1978.
- 11 Lefer AM, Sollott SL, Galvin MJ: Beneficial actions of prostacyclin in traumatic shock. *Prostaglandins* 17:761-767, 1979.

- 12 Feuerstein N, Ramwell PW: Effects of endotoxin on prostaglandin release from rat lung. *Br J Pharmac* 73:511-516, 1981.
- 13 Jacobs ER, Soulsby ME, Bone RC, Wilson FJ Jr, Hiller FC: Ibuprofen in canine endotoxin shock. *J Clin Invest* 70:536-541, 1982.
- 14 Romson JL, Bush LR, Haack DW, Luechesi BR: The beneficial effects of oral ibuprofen on coronary artery thrombosis and myocardial ischemia in conscious dog. *J Pharmacol Exp Ther* 215:271-278, 1980.
- 15 Abrahamsen AF: A modification of the technique for ⁵¹Cr-labelling of blood platelets giving increased circulating platelet radioactivity. *Scand J Haemat* 5:53-63, 1968.
- 16 Brigham KL, Bowers RE, Haynes J: Increased sheep lung vascular permeability caused by *Escherichia coli* endotoxin. *Circ Res* 45:292-297, 1979.
- 17 Heideman M, Kaijser B, Gelin LE: Complement activation early in endotoxin shock. *J Surg Res* 26:74-78, 1979.
- 18 Smith ME, Gunther R, Gee M, Flynn J, Demling RH: Leukocytes, platelets and thromboxane A₂ in endotoxin-induced lung injury. *Surgery* 90:102-107, 1981.
- 19 Jacob HS, Moldow CF, Flynn PJ, Weisdorf DJ, Vercellotti GM, Hammerschmidt DE: Therapeutic ramifications of the interaction of complement, granulocytes, and platelets in the production of acute lung injury. *Ann N Y Acad Sci* 384:489-495, 1982.
- 20 Kessler E, Huges RC, Bennett EN, Nadela SM: Evidence for the presence of prostaglandin-like material in the plasma of dogs with endotoxin shock. *J Lab Clin Med* 81:85-94, 1973.
- 21 Spagnuolo PJ, Ellner JJ, Hassid A, Dunn MJ: Thromboxane A₂ mediates augmented polymorphonuclear leukocyte adhesiveness. *J Clin Invest* 66:406-414, 1980.
- 22 Smith IB, Araki H, Lefer AM: Thromboxane A₂, prostacyclin and aspirin: effects on vascular tone and platelet aggregation. *Circulation* 62:19-25, 1980.

- 23 Demling RH, Smith MS, Gunter R, Gee M, Flynn J: The effect of prostacyclin infusion on endotoxin-induced lung injury. *Surgery* 89:257-263, 1981.
- 24 Kraus MK, Utsunomiya T, Dunham B, Valteri R, Shepro D, Hechtman HB: Inhibition of permeability edema with imidazole. *Surgery* 92:299-308, 1982.
- 25 Ogletree ML, Brigham KL: Indomethacin augments endotoxin induced lung vascular permeability in sheep. *Am Rev Respir Dis* 119 (Suppl):383, 1979.

TABLE 1

Blood pressure decrease from baseline values (mm Hg)

Group	n	5 min	120 min
		Mean $\pm$ SD	Mean $\pm$ SD
Sham	5	0	10 $\pm$ 8
I-C	5	0	0
Ex-C	5	61 $\pm$ 25*	74 $\pm$ 30*
Ex-I	4	61 $\pm$ 36*	23 $\pm$ 23
I-Ex	4	64 $\pm$ 40*	18 $\pm$ 13

* Significantly different from sham

TABLE II

Pulmonary platelet trapping, HCT, platelet and WBC counts (Mean $\pm$ SD)

Group	n	PPT (^{51}Cr Lung/Blood)	HEMATOCRIT (Increase over baseline value)	PLATELETS (% of Baseline Counts)		WHITE BLOOD CELLS (% of Baseline Counts)	
				120 min	10 min	10 min	120 min
Sham	4	$1.30 \pm 0.28^*$	1	(100)	97 ± 7	(100)	102 ± 2
I-C	5	1.31 ± 0.24	1	95 ± 12	92 ± 12	95 ± 10	92 ± 9
Ex-C	5	$3.76 \pm 1.43^+$	$21 \pm 4^+$	$13 \pm 12^+$	$24 \pm 11^+$	$6 \pm 4^+$	$27 \pm 13^+$
Ex-I	4	$1.93 \pm 0.12^{+0}$	$14 \pm 7^+$	$6 \pm 2^+$	59 ± 28	$11 \pm 5^+$	$46 \pm 25^+$
I-Ex	4	1.63 ± 0.26^0	$18 \pm 9^+$	$6 \pm 8^+$	55 ± 26	$20 \pm 18^+$	87 ± 30^0

* n = 5

+ Significantly different from sham control

0 Significantly different from endotoxin control

PAPER IV

PULMONARY PLATELET TRAPPING INDUCED BY β -ENDORPHIN
INJECTION IN THE CEREBROSPINAL FLUID IN DOGS.

Per M Almqvist, Karl M Knigge, Marian Kuenzig, Shirley A
Joseph, Webster Pilcher and Seymour I Schwartz.

Department of Surgery and The Neuroendocrine Unit,
University of Rochester, School of Medicine and Dentistry,
Rochester, N.Y., USA

Correspondence to:

Dr Per M Almqvist

c/o S I Schwartz

Dept Surgery

Strong Memorial Hospital

601 Elmwood Avenue

Rochester, N.Y. 14642

USA

ABSTRACT

The effect of β -endorphin injected either into the lateral ventricle or the cisterna magna, on blood pressure, heart rate, peripheral platelet and leucocyte counts, hematocrit, catecholamines and pulmonary platelet trapping was studied. The effect of endotoxin on the endogenous opiocortin system was also investigated. Injection of β -endorphin caused a significant hypotension, bradycardia and pulmonary platelet trapping. It had no effect on peripheral platelet and leucocyte counts, catecholamines or hematocrit. Endotoxin shock caused a marked rise in circulating β -endorphin and a decrease in cerebrospinal fluid β -endorphin. Our results indicate that endotoxin shock stimulates hypothalamic opiocortin neurons to increased secretory activity. We suggest that the endorphins may participate in the evolution of the lung injury seen in septic shock.

INTRODUCTION

Since the discovery of opiate receptors (Terenius 1973) and their natural ligands in the rat brain (Hughes 1975) the endogenous opiates have generated much research on their role as neurotransmitters and as participants in several physiological systems (11, 20). Increased levels of circulating β -endorphin have been shown in rats subjected to trauma and to peripheral endotoxin injection (8, 23). The role of endorphin as a mediator in shock was proposed by Holaday and Faden who found that the opiate antagonist, naloxone, significantly improved hypotension in endotoxin shocked rats (10). Since then it has been shown that naloxone is also effective in restoring blood pressure in hemorrhagic shock both in rats and in dogs (7). In addition to its effect on hypotension naloxone treatment has been reported to attenuate acidosis and hypoglycemia in endotoxin shocked dogs (17).

Trapping of platelets in the pulmonary vasculature is believed by many to be involved in the etiology of the lung injury associated with different types of shock. We have shown naloxone effective in protecting against pulmonary platelet trapping (PPT) in dogs subjected to endotoxin and to trauma. In the same experiments we also confirmed the positive effects of naloxone on hypotension (3, 4).

The present experiment was done to determine whether centrally administered β -endorphin would induce shock and pulmonary injury in dogs and whether naloxone treatment would obviate such effects. The response of the dogs own opiocortin system to injection of exogenous β -endorphin either into the lateral ventricle or into the cisterna magna and to endotoxin shock was also an objective of this study.

MATERIAL AND METHODS

Sixteen adult mongrel dogs of both sexes weighing between 9-14 kg were used. None of these dogs had diarrhea, white blood cell counts over $17\,000/\text{mm}^3$ or obvious respiratory infection.

One day prior to the shock study the dogs were anesthetized with pentobarbital sodium 30 mg/kg b.w., endotracheally intubated and connected to a Harvard respirator. The respiration rate was 12 per minute and the volume was 20 cc per kg b.w. plus allowance for dead space. 100 ml of blood was drawn from each dog, the platelets were tagged with ^{51}Cr according to a modification of a method described by Abrahamsen (1), and the autologous platelets were reinfused. On the following day the dogs were reanesthetized and placed on a specially constructed board with a head holding device. A plastic catheter was inserted in the femoral artery and connected to a pressure gauge for measurement of peripheral blood pressure. A three-way stopcock was inserted in the line for blood sampling. The head and neck were shaved, a skin incision was made over the sagittal suture and the temporal muscle of one side partially retracted from its origin. A drill hole was made in the parietal bone 10 mm posterior to bregma and 10 mm lateral to the sagittal suture. An 18 gauge spinal needle with a stylet was inserted until cerebrospinal fluid from the lateral ventricle was obtained, and then capped. The needle was fixed to the bone with glue (Zipbond, Tescom Corp., Minneapolis, Minn.). An 18 gauge spinal needle was introduced into the cisterna magna and connected by a three-way stopcock to a central venous pressure manometer for continuous measurement of intracranial pressure and for sampling.

Human β -endorphin (Lipotropin 61-91, Peninsula Lab., San Carlos, CA) at a dose of 50 μ g/kg b.w. in 0.5 ml normal saline was injected over a one-minute period either into the lateral ventricle or into the cisterna magna. Control dogs were injected with 0.5 ml NaCl.

Blood pressure and intracranial pressure were measured continuously during the experiment. The lungs were hyperinflated periodically to prevent atelectasis. Arterial blood samples were taken prior to β -endorphin injection for baseline values of Hct, peripheral platelet and WBC counts, plasma β -endorphin, catecholamine (dopamine, epinephrine and norepinephrine) and ^{51}Cr activity. Blood sampling for Hct, platelet and WBC counts was repeated hourly; at 5, 10, 30, 60, 90 and 120 minutes for catecholamine measurements; and for β -endorphin radioimmunoassay (RIA) determinations at 10, 30, 60, 120, 180, 240 and 300 minutes after β -endorphin injections. At five hours a blood sample was taken for ^{51}Cr activity. Cerebrospinal fluid samples (CSF) were taken at the same time as the blood samples for β -endorphin (RIA). No ten-minute (CSF) sample was taken from the cisterna magna injected dogs. Hematocrit was measured in duplicate in microhematocrit tubes. Blood was drawn into Unopettes (Becton Dickinson Co., Rutherford, N.J.) and WBCs and platelets were counted in a counting chamber under light microscopy. Blood for the catecholamine determinations was drawn into EGTA solution and assayed by a commercially available RIA kit (Cat-A-Kit, the Upjohn Co., Kalamazoo, Mich.). For β -endorphin, 3 ml of blood was collected in ACD coated glass tubes and 350 μ l CSF was collected in plastic tubes; the samples were immediately placed on ice.

At sacrifice the lungs were excised, weighed and dried with pressurized air for 5-7 days. The pleura was peeled off the dried lungs to avoid counting surface blood activity, and the lung parenchyma was divided and placed in tubes.

^{51}Cr activity of the blood and lung was counted in a gamma scintillation counter. The ratio of counts/g lung tissue to counts/g blood was determined and used as a measure of PPT. The wet/dry lung weight ratio was calculated and used as a measure of pulmonary edema. Immediately after sacrifice the brain and the pituitary were removed and placed on dry ice. These tissues were stored at -40°C for later studies.

Endorphins were extracted with talc from 2 ml aliquots of thawed plasma according to the procedure of Wardlaw and Frantz (22); extractions were carried out in 15 x 100 mm siliconized tubes. Recovery of β -endorphin standards was $84 \pm 6\%$. Aprotinin, 0.5 trypsin inhibitor units (Trasylol^R) was added to each cooled CSF sample before it was frozen. CSF samples required no other treatment or extraction prior to RIA. CSF and extracted plasma samples were divided into 2 aliquots; β -endorphin was assayed using antisera generated against either camel or human β -endorphin, by conventional RIA.

Four groups of 4 dogs each were studied; Group I, nonshocked dogs injected with 0.5 cc normal saline either into the lateral ventricle (2 dogs) or the cisterna magna (2 dogs); Group II, human β -endorphin injected into the lateral ventricle; Group III, human β -endorphin injected into the cisterna magna; Group IV; β -endorphin injected either into the lateral ventricle (2 dogs) or into the cisterna magna (2 dogs) plus intravenous injection of naloxone 2 mg/kg b.w. 10 min before and every 30 min after human β -endorphin injection. In addition, in 5 dogs injected with endotoxin, CSF (2 dogs), blood (3 dogs) and brain (3 dogs) endorphin-ir was assayed. These dogs did not have a needle in the lateral ventricle, the platelets were not tagged and they were studied for two hours only.

Statistical significance was tested with Wilcoxon's rank sum test; a p-value of less than 0.05 was considered significant.

RESULTS

Blood pressure: Blood pressure was stable in the sham control (Group I).

In Group II (intraventricular injection of human β -endorphin) there was a small increase by 5 min; blood pressure returned to control levels by 30 min and thereafter gradually decreased. By 3-4 hours blood pressure was significantly lower than in control dogs. In Group III (intracisternal human β -endorphin injection) hypotension was evident at 10 min and significant at 120 min and was present throughout the 5-hour period. The 5-hour drop from baseline was 24 ± 9 mmHg for Group II and 35 ± 20 for Group III; the difference between the end values of these two groups is not statistically significant. Naloxone treatment obviated the blood pressure changes caused by β -endorphin injection either into the cisterna magna or into the lateral ventricle (fig 1).

Heart rate: Group I showed a gradual decrease beginning after 1 hour; the mean decrease at 5 hours was 23 ± 5 beats/min (Table I). Group II had an initial increase which lasted approximately 15 min followed by a decrease which was already significantly lower than control at 30 min; the mean drop after 5 hours was 56 ± 27 beats/min. Group III had an immediate decrease in heart rate which was significantly different compared to control at 30 min and continued to drop; the decrease from baseline at 5 hours was 73 ± 33 which is not significantly different from Group II. Group IV showed heart rate changes similar to sham; the mean drop at 5 hours was 15 ± 10 .

Intracranial pressure: There was no change in Group I during 5 hours. Group II and Group III had a slight increase, range 2-9 cm H₂O, this increase was not alleviated by naloxone treatment.

Hematocrit: There were no significant changes in any of the groups during the experiment (Table II).

Peripheral platelet counts: The peripheral platelet counts were stable throughout the 5-hour study in all the groups (Table II).

Peripheral white blood cell count: There were no significant changes in Groups I, III and IV. Group II showed an increase over baseline at 5 hours (Table II).

Wet/dry lung weight ratio: This parameter was slightly, but not significantly higher in both Groups II and III compared to Group I. Group IV had a normal ratio (Table III).

Catecholamines: The variation in serum levels did not differ between any of the groups.

⁵¹Cr lung/blood ratios: Groups II and III had ratios higher than Group I indicating that β -endorphin increased PPT. This increase was statistically significant for Group II. Group IV had a normal ratio reflecting prevention of the β -endorphin mediated PPT by naloxone (Table III).

Endorphins in brain, CSF and blood

Table IV defines the cross-reactivity of the two antisera used in this study with various peptides of the opiocortin family. Antiserum β E-4, raised against camel β -endorphin (c β E) is fairly specific for c β E, the sensitivity is 100%. Of the larger molecular weight components, it recognizes only human (h) β -lipotropin to any significant degree. Antiserum β E-1 raised against human β -endorphin (h β E), also has a 100% sensitivity, it cross-reacts quite broadly.

CSF β E-immunoreactivity (ir): The basal concentration of β -endorphin-ir in CSF varied between dogs ranging from 0 to 200 ng/ml for human β -endorphin-ir and from 0 to 20 ng/ml for camel β -endorphin-ir. Following injection of h β E into the lateral ventricle or cisterna magna, h β E-ir was measured at 1500 - 5500 ng/ml 30 min after injection; it declined over the 5-hour experimental period to values in the range of 370 to 1700 ng/ml. In the presence of these high levels of exogenous h β E and because of the cross-reactivity of the anti-camel β E antiserum (1%) with h β E, it was not possible to accurately assess the response of the dog's endogenous c β E-ir. Naloxone treatment did not alter the levels of the injected h β E or the rate at which it disappeared from the CSF.

Plasma β -endorphin-ir: In control dogs, c β E-ir ranged between 200 and 1700 pg/ml during the 5-hour experiment period. No detectable h β E-ir was found. Following injection of h β E into the cisterna magna, high concentrations of h β E-ir (30-2200 pg/ml) were present in plasma within 30 minutes and were sustained in this range or increased further during the 5-hour period (650-2700 pg/ml). Injection of h β E into the lateral ventricle resulted in a more gradual appearance of h β E-ir in plasma; h β E-ir appeared at low values

between 30 minutes and 2 hours, increasing to 300-1000 pg/ml by 5 hours. As in the case of CSF, it was not possible to measure endogenous c β E-ir in these h β E-treated dogs.

Endotoxin induced a marked fall in CSF c β E-ir which remained low during the 2-hour study. Endotoxin injection caused a three to five fold increase in plasma c β E-ir and an increase in plasma h β E-ir from 0 to 400-2000 pg/ml.

Brain β E-ir: Chromatographic separation and assay (6) for c β E-ir and h β E-ir in tissue of the mediobasal hypothalamus representing the location of opicortin cell bodies indicate that in control animals, the majority of β E-ir is in the small molecular weight "endorphin" zone and is recognized only with c β E antiserum. After 2 hours of endotoxin shock, there is a reduction in the amount of c β E-ir accompanied by the appearance of h β E-ir.

DISCUSSION

β -endorphin is a member of the opiocortin family of peptides (6). It has been located in the pituitary gland, the hypothalamus and the brain stem (13, 14). The anatomic location strongly suggests involvement of the opiocortins with autonomic centers and with brain stem pathways involved in the transmission of pain (12). The current literature contains many reports describing the increase of plasma endorphins in various stress situations. Fracture of the tibia and fibula in rats results in an increase of both β -endorphin and ACTH in blood and this is not seen in hypophysectomised rats nor in rats pretreated with steroids (8). A similar response is seen in rats subjected to endotoxin injection or to electrical footshock (19, 23). Boarder recently found that mild stress resulting from 2 minutes of knee bend exercise increased plasma

opiate activity two-fold in man (5). It is now commonly accepted that stress causes a release of endorphin from the pituitary.

Holaday and Faden were the first to propose that blocking the endorphins might be helpful in shock. They treated endotoxin shocked rats with naloxone, an opiate receptor blocker, and found a beneficial effect on blood pressure. However, in this model it did not increase the survival rate (10). To determine if this response was specific to the endotoxin shock model or was species specific they conducted further trials with hemorrhagic shock in both rats and dogs and with endotoxin shock in dogs. They found similar positive effects on hypotension and, in addition, increased survival rate in these later experiments (7). These findings were later confirmed by other investigators who also showed naloxone effective in reducing the acidosis and hypoglycemia of experimental endotoxin shock (2, 9, 17). These naloxone studies provide indirect evidence that β -endorphin is released during shock and participates in some of its pathophysiological events. There have been several case reports concerning the use of naloxone in different shock states in humans where naloxone has been shown to be effective in improving hypotension refractory to traditional shock treatment (16, 21).

It is currently thought that the endorphins have a central action and this viewpoint is supported by the fact that centrally administered naloxone in concentrations ineffective when given systemically, have a protective effect on hypotension in dogs subjected to endotoxin shock (12). However, opiate receptors have also been found in the heart, lung, intestine, and the adrenals (15), and this suggests that endorphins may also act peripherally. β -endorphin, when injected into an isolated segment of dog stomach, caused an injury to the epithelial cells similar to that seen when live bacteria were injected

via the same route. Naloxone prevented this cell injury and attenuated the acidosis produced by live bacteria (18). This provides support for peripheral action of β -endorphin.

In earlier work we have shown naloxone effective in reducing pulmonary platelet trapping in dogs subjected to endotoxin injection and to completely obviate PPT caused by soft tissue trauma (3, 4). Those results provided indirect evidence suggesting a role for endorphins as mediators of the pulmonary injury in shock and in trauma. The present study was done to obtain more direct evidence for this theory. We were able to show that a bolus dose of human β -endorphin injected either into the lateral ventricle or into the cisterna magna caused a profound bradycardia and also produced hypotension which was more pronounced when β -endorphin was injected into the cisterna magna. The intraventricular injection caused an initial rise (not statistically significant) in both heart rate and blood pressure which was not seen in the intracisternally injected dogs. This may imply that the endorphin injected into the cerebral ventricle acts upon blood pressure regulating hypothalamic centers, after a transit time of 7-10 min endorphin reaches the medullary autonomic center causing hypotension. The hypotension seen in this experiment with a high dose of injected β -endorphin is much less than that seen when endotoxin is injected in dogs, suggesting that the hypotension due to endotoxin injection is not mediated by endorphins alone. Our previous results support this view since naloxone treatment of the endotoxin shock model, while improving hypotension, did not return the blood pressure to control values. The present experiment also demonstrated that centrally administered β -endorphin causes pulmonary platelet trapping. This effect, and also the hypotension and bradycardia, were naloxone reversible.

It is assumed that the injected β -endorphin activated the same neural systems which are engaged by the endogenous opiocortin system during endotoxin shock. The present study offers some insight into the possible changes in activity of the hypothalamic opiocortin system during endotoxin shock. The characteristics and cross-reactivities of our two β -endorphin antisera suggest that β E-4 (anti camel β -endorphin) is recognizing the β -endorphin endogenous to the dog and that β E-1 (anti h β E) recognizes the larger molecular precursors, (Table IV). Following endotoxin, there occurs in the CSF an abrupt fall in c β E-ir. In the hypothalamic opiocortin system the kinetics of opiocortin biosynthesis appears to be normally set so that the final product (β -endorphin) occupies the largest compartment, with undetectable amounts of intermediates or precursors. Following endotoxin injection large amounts of h β E-ir intermediates appear and the concentration of c β E-ir declines. These observations may suggest that endotoxin shock stimulates hypothalamic opiocortin neurons to increased secretory activity with more rapid movement of final product to the projection fields of this system. The fall in concentration of camel β E-ir in CSF during shock, in spite of increased synthesis, may be related to altered receptor activity or to inactivation. The prompt rise in plasma c β E-ir following endotoxin injection reflects the well recognized activation of the pituitary-adrenal axis, with release of ACTH and β E from the adenohypophysis. The appearance in plasma of h β E-ir, as in the case of the central opiocortins, may reflect the activation of this system in the pituitary, with overflow of intermediate products into the plasma. The question of whether this plasma β E enters the brain and is a factor in central events in shock remains to be investigated. This issue may be of some importance, since the perikarya of the hypothalamic and medullary opiocortin systems are located in areas which have no blood-brain barrier and the β E in plasma thus has access to the interstitial fluid surrounding these neurons.

In conclusion, we have shown that exogenous β -endorphin, administered centrally, induces hypotension, bradycardia and pulmonary platelet trapping; endotoxin activates the opiocortin system and increased amounts of β -endorphin are seen in the systemic circulation. We suggest that β -endorphin may participate in the evolution of the lung injury associated with septic shock.

1. Abrahamsen, A.F. A modification of the technique for ^{51}Cr -labelling of blood platelets giving increased circulating platelet radioactivity. *Scand J Haemat* 5:53-63, 1968.
2. Albert, S.A., G.T. Shires, III, H. Illner and G.T. Shires. Effects of naloxone in hemorrhagic shock. *Surg Gynecol Obst* 155:326-332, 1982.
3. Almqvist, P.M., M. Kuenzig and S.I. Schwartz. Effect of naloxone on endotoxin induced pulmonary platelet sequestration. *Surgical Forum* 32:304-306, 1981.
4. Almqvist, P.M., M. Kuenzig and S.I. Schwartz. The effect of naloxone and cyproheptadine on pulmonary platelet trapping, hypotension, and platelet aggregability in traumatized dogs. *J Trauma* 23:405-407, 1983.
5. Boarder, M.R., E. Erderlyi and J.D. Barchas. Opioid peptides in human plasma: Evidence for multiple forms. *J Clin Endocrin Met* 54:715-720, 1982.
6. Eipper, B. and R. Mains. Structure and biosynthesis of proadrenocorticotropin/endorphin and related peptides. *Endocrine Rev* 1:1-23, 1980.
7. Faden, A.J. and J.W. Holaday. Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. *J Infect Dis* 2:229-238, 1980.
8. Guillemin, R., T. Vargo, J. Rossier, S. Minick, N. Ling, C. Rivier, W. Vale and F. Bloom. β -endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 197:1367-1369, 1977.

9. Gurll, N.J., T. Vargish, D.G. Reynolds and R.B. Lechner. Opiate receptors and endorphins in the pathophysiology of hemorrhagic shock. *Surgery* 89: 364-369, 1981.
10. Holaday, J.W. and A. J. Faden. Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. *Nature* 275:450-451, 1978.
11. Hughes, J., T.W. Smith, H.W. Kosterlitz, L. Fothergill, B.A. Morgan and H.R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258:577-579, 1975.
12. Janssen, H.F. and L.O. Lutherer. Ventriculocisternal administration of naloxone protects against severe hypotension during endotoxin shock. *Brain Res* 194:608-612, 1980.
13. Knigge, K.M. and S.A. Joseph. Anatomy of the opioid-systems of the brain. *Canad J Neurol Sci* 11:14-23, 1984.
14. Liotta, A., D. Gildersleeve, M. Brownstein and D. Krieger. Biosynthesis in vitro of immunoreactive 31,000 dalton corticotropin/ β -endorphin-like material by bovine hypothalamus. *Proc Natl Acad Sci, USA* 76:1448-1452, 1979.
15. Neidle, A., J. Manigarlil and I.J. Wajda. Distribution of opiate-like substances in rat tissues. *Neurochemical Res* 4:399-410, 1979.
16. Peters, W.P., M.W. Johnson, P.A. Friedman and W.E. Mitch. Pressor effect on naloxone in septic shock. *Lancet*, March 7, 1981.

17. Raymond, R.M., J.M. Harkema, W.V. Stoffs and T.E. Emerson. Effects of naloxone therapy on hemodynamics and metabolism following a superlethal dosage of *Escherichia coli* endotoxin in dogs. *Surg Gynecol Obst* 152:159-162, 1981.
18. Rees, M., J.G. Payne and J.C. Bowen. Naloxone reverses tissue effects of live *Escherichia coli* sepsis. *Surgery* 91:81-86, 1982.
19. Rossier, J., E.D. French, C. Rivier, N. Ling, R. Guillemin and F. Bloom. Foot-shock induced stress increased β -endorphin levels in blood but not in brain. *Nature* 270:618-620, 1977.
20. Terenius, L. Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. *Acta Pharmacol et Toxicol* 33:377-384, 1973.
21. Tiengo, M. Naloxone in irreversible shock. *Lancet*, Sept. 27, 1980.
22. Wardlaw, S. and A. Franz. Measurement of β -endorphin in human plasma. *JCEM* 48:176-180, 1979.
23. Yamazaki, N., E. Okabe, A. Hara, K. Takahashi and H. Ito. Pharmacological study on endotoxin shock. IIth Europ Conf Microcirculation. Basel, Karger 1980, pp 472-476.

Fig. 1

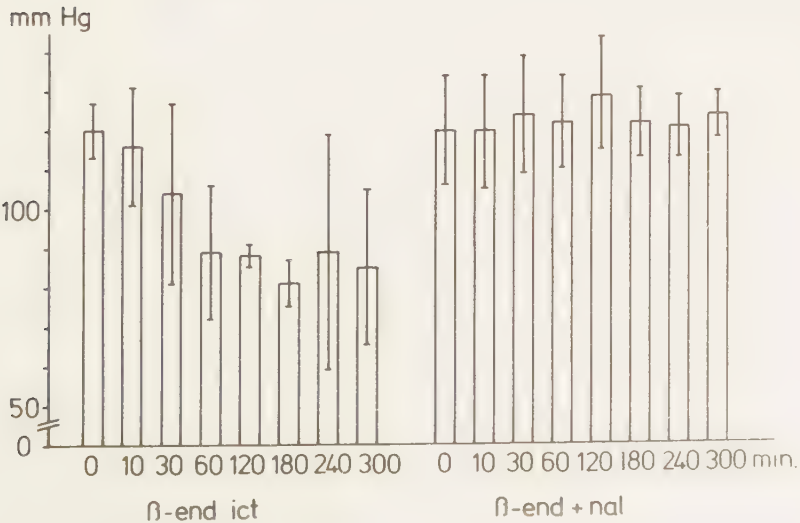
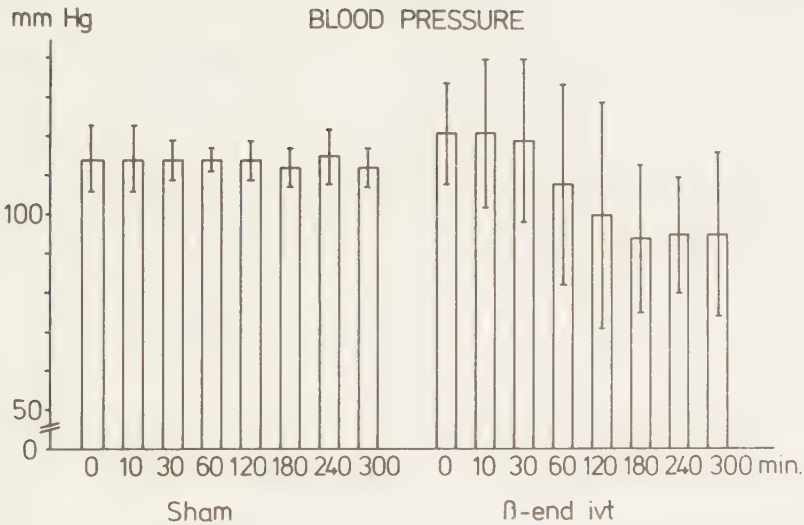


TABLE I

HEART RATE

	<u>n</u>	<u>0 min</u>	<u>60 min</u>	<u>300 min</u>
control	4	120 [±] 6	120 [±] 8	100 [±] 8
β-end i.v.t.	4	115 [±] 10	66 [±] 6*	64 [±] 20
β-end i.c.t.	4	130 [±] 16	72 [±] 22*	53 [±] 16*
β-end + nal	4	125 [±] 19	118 [±] 30	110 [±] 28

* Significantly different compared to control p<0.05

TABLE II

BLOOD VALUES

Group	n	Hct		Platelet counts		WBC counts	
		0	5 h	0	5 h	0	5 h
Control	4	34 [±] 6	34 [±] 4	275,000	275,000	9,000	9,100
				[±] 50,000	[±] 50,000	[±] 3,500	[±] 3,400
I.v.t. β -end	4	37 [±] 7	36 [±] 5	292,000	293,000	6,100	11,100
				[±] 55,400	[±] 114,400	[±] 2,800	[±] 3,000
I.c.t. β -end	4	32 [±] 5	31 [±] 6	244,000	230,000	11,200	11,000
				[±] 38,600	[±] 46,500	[±] 4,800	[±] 4,500
β -end + nal	4	34 [±] 9	36 [±] 9	236,000	241,000	11,200	10,600
				[±] 38,200	[±] 20,200	[±] 5,200	[±] 3,200

All values are mean $\pm$ S.D.

TABLE III Indication of PPT and pulmonary edema

Group	n	^{51}Cr lung/blood mean $\pm$ S.D.	wet/dry lung wt mean $\pm$ S.D.
Control	4	1.35 $\pm$ 0.25	4.7 $\pm$ 0.2
I.v.t. β -end	4	1.95 $\pm$ 0.47*	5.0 $\pm$ 0.2
I.c.t. β -end	4	1.66 $\pm$ 0.52	4.9 $\pm$ 0.3
β -end + nal	4	1.24 $\pm$ 0.18	4.8 $\pm$ 0.2

* Significantly different from sham $p < 0.05$

TABLE IV Cross-reactivity of antibodies β E-1 and β E-4
with opioꝛortin peptides

Peptide	Percent cross-reactivity with antibody	
	β E-4 (anti c β E)	β E-1 (anti h β E)
Camel β -endorphin	-	17
Human β -endorphin	1.0	-
Human β -lipotropin	20	100
Porcine β -lipotropin	0.01	13
Ovine β -lipotropin	0.72	11
31K Precursor (Li)	1.4	86

PAPER V

β -ENDORPHIN ADMINISTERED INTO THE LATERAL VENTRICLE OF THE
BRAIN CAUSES PULMONARY PLATELET TRAPPING IN RABBITS

Per Almqvist, Carin Larsson-Backström, Lisbet Nilsson &
Ulf Haglund

Department of Surgery, University of Lund, Malmö General
Hospital, Malmö, Sweden.

Address correspondence and proofs to

Ulf Haglund, M.D., Department of Surgery, Malmö General
Hospital, S-214 01 MALMÖ, Sweden

SUMMARY

Human β -endorphin was injected into the cerebrospinal fluid in rabbits by means of a needle inserted into the lateral ventricle of the brain. Control rabbits received an equal amount of saline. β -endorphin induced a significant pulmonary platelet trapping compared to control. β -endorphin had no effect on arterial blood pressure, heart rate, platelet aggregability *ex vivo* or fibrinolytic activity (fibrinolytic plates). The plasma activity of antithrombin-III, kallikrein like activity and kallikrein inhibitor determined by means of chromogenic substrates was not influenced by β -endorphin.

Key words

Rabbits, platelet, pulmonary trapping, endorphin, central action

Increased levels of circulating β -endorphin have been demonstrated in rats and dogs following traumatic and endotoxic shock (1-3). A possible role for endogenous opiates in the pathophysiology of shock has been suggested on the basis of the demonstration that naloxone, an opiate antagonist, prevents hypotension and significantly improves metabolic status in animals subjected to shock inducing challenges (4-7). In dogs subjected to soft tissue trauma or endotoxin shock naloxone protects against pulmonary platelet trapping (8, 9). Recent experiments have shown that administration of human β -endorphin into the cerebrospinal fluid of dogs induced pulmonary platelet trapping, hypotension and bradycardia which all were blocked by intravenous naloxone (10). It was suggested that activation of endogenous opiates in shock could be an important factor in the development of ARDS following septic shock.

This series of experiments was performed to further analyse the role of endogenous opiates in pulmonary platelet trapping and hence possibly the development of adult respiratory distress syndrome (ARDS) in shock. More specifically the aim was to study whether the effect of centrally administered β -endorphin could be repeated in another species of animals and, if so, if the increased pulmonary trapping of platelets was associated with any changes in the plasma humoral cascade systems or in platelet aggregability.

MATERIAL AND METHODS

Twenty-eight rabbits of both sexes, weighing between 2.5 and 6 kg were used. The animals were without obvious signs of respiratory or intestinal diseases. The rabbits were anaesthetized with pentobarbital (Mebumal Vet., 60 mg/ml, ACO Läkemedel, Sweden) 15 mg/kg/b.w. i.v. and repeated doses of 15 mg were given to maintain adequate level of anaesthesia. Tracheostomy was performed and the rabbits were connected to a respirator (B. Braun Melsungen AG) set at 2.5 ml/kg/b.w. and a frequency of 40 breaths/min. A plastic catheter was inserted in the femoral artery for blood pressure and heart rate measurements and blood sampling. The head was shaved and a skin incision made over the sagittal suture. A drill hole was made approximately 10 mm posterior to the bregma and 10 mm lateral to the sagittal suture. A plastic catheter with a stilette was inserted until cerebrospinal fluid from the lateral ventricle was obtained and then capped. The catheter was fixed to the bone with glue (Loctite, cyanoacrylate, 424, G.A. Lindberg, Stockholm).

Human β -endorphin 50 μ g/kg/b.w. (Lipotropin 61 - 91, Bachem Feinchemikalien AG, Bubendorff, Switzerland) in 0.2 ml NaCl was injected slowly over a one-minute period into the lateral ventricle. The rabbits were studied for three hours, then sacrificed with an overdose of pentobarbital. Blood pressure and heart rate were measured continuously during the experiment. Controls were given equal amounts of saline. Arterial blood samples were taken prior to β -endorphin

injection to obtain baseline values of platelet aggregability, fibrinolytic activity, antithrombin III, kallikrein like activity, kallikrein inhibitor. The blood for these analyses were taken into sodium citrated glass tubes. The chromogenic substrate samples were stored at -20°C and all samples were assayed on the same days. Serum samples for determination of fibrin degradation products (FDP) were stored in plastic tubes at -20°C after addition of trombin, NaCl and EACA as described by Nilsson (11). Blood samples for platelet aggregability were taken 10 and 180 min after β -endorphin injection while blood samples for the other parameters were taken 10, 30, 60, 90; 120, 150 and 180 min after β -endorphin injection.

Fibrinolytic activity was measured with the fibrin plate method (12). The ex vivo platelet aggregability to ADP (10^{-4}M) and collagen ($20\text{ }\mu\text{g/ml}$) was measured in a platelet aggregation profiler (Model PAP-3, Bio/Data Corp. Horsham, Pa 19044). The maximal slope in mm/min was calculated as a measure of inhibition or stimulation of platelet aggregability.

The concentration of antithrombin III, kallikrein like activity and kallikrein inhibitor was measured with chromogenic peptide substrates (13, 14) obtained from Kabi Diagnostica AB, Stockholm, Sweden.

Five groups of rabbits were studied:

Group I: Control experiments; 0.2 ml NaCl injected into the lateral ventricle (i.v.t.).

Group II: β -endorphin in 0.2 ml NaCl (i.v.t.).

Group III: β -endorphin in 0.2 ml NaCl (i.v.t.) plus naloxone 1 mg/kg/b.w. intravenously 10 min before β -endorphin injection and repeated every 30 minutes thereafter.

Pulmonary platelet trapping (PPT) was studied in two separate groups:

Group IV: 0.2 ml NaCl (i.v.t.) and

Group V: β -endorphin in 0.2 ml NaCl (i.v.t.).

Homologous platelets were tagged with ^{51}Cr according to a method described by Abrahamsen (15). This was performed one day before the experiment and the tagged platelets were given to the experimental animal that day. 25 ml whole blood was taken via heart puncture from each donor rabbit. Platelets from four donor rabbits were pooled and the radioactively labelled platelets were later given to two rabbits. A total volume of about 6 ml was given to each animal.

Immediately before sacrifice a blood sample was taken and at sacrifice one lung was taken out. The lung was dried, divided and placed into tubes. ^{51}Cr activity of the blood and the lung was counted in a gamma-scintillation counter (LKB Wallace, 1280 Ultro gamma). The ratio of

counts/g lung tissue to counts/g blood was calculated and used as a measure of PPT.

Data are expressed as mean values $\pm$ SD. The statistical analysis was done with the Wilcoxon's rank sum test for unpaired data and the one tailed Mann-Whitney U-test for comparing the ^{51}Cr lung/blood ratios. A p-value of less than 0.05 was considered significant.

RESULTS

Blood pressure and heart rate were stable throughout the observation period in the control group. β -endorphin caused no significant drop in blood pressure and had no effect on heart rate (Fig 1 and Table I).

The ^{51}Cr lung/blood ratio was significantly higher in the β -endorphin injected rabbits compared to controls (0.87 ± 0.38 vs 0.48 ± 0.15 ; $p < 0.05$). Fibrinolytic activity was not significantly different between the groups, but there was a decrease in the group that received both β -endorphin and naloxone (Group III) (Table II). There were no measurable fibrin split products in any of the groups.

Antithrombin III, kallikrein like activity and kallikrein inhibitor activity did not differ significantly between the groups (Table III). The in vitro platelet aggregability to ADP and collagen did not differ significantly between any of the groups (Table IV).

DISCUSSION

This series of experiments demonstrates that β -endorphin injected into the lateral ventricle of the brain causes pulmonary platelet trapping in rabbits. This confirms an earlier, yet unpublished, observation that β -endorphin injected into the cerebrospinal fluid of dogs induces pulmonary trapping of platelets (10). In that series of experiments β -endorphin (lipotropin 61-91, Peninsula Lab, San Carlos, CA; 50 μ g/kg) injected into the lateral ventricle or the cisterna magna caused PPT, systemic hypotension and reduced heart rate compared to controls which received an equivalent amount of saline (Almqvist, personal communication). Furthermore, the effects of β -endorphin was abolished if naloxone (2 mg/kg) was given i.v. before, and repeatedly every 30 min after β -endorphin. There was no significant effects on blood pressure or heart rate in the present studies on rabbits. This might indicate a species difference. It should, however, be kept in mind that the endorphin given is human β -endorphin. There are today two commercially available forms of β -endorphin; human and camel. The lack of effects on systemic arterial blood pressure and heart rate in this study could possibly be due to the fact that human β -endorphin is not "physiological" for rabbits.

Centrally administered β -endorphin could induce pulmonary platelet trapping by different mechanisms acting independently or in cooperation. In dogs it has been found that the

increased pulmonary platelet trapping was not associated with any increase in circulating catecholamines (Almqvist, personal communication). Another possibility tested in this study is an effect on the circulating platelets and/or on plasma fibrinolytic activity. However, there was no change in platelet aggregability as induced by ADP or collagen nor in plate fibrinolysis ex vivo or in fibrin split products in plasma. The reduction in fibrinolytic activity found at 3 h in the group given β -endorphin in the lateral ventricle of the brain and i.v. naloxone (Table II) is not possible to explain. However, the effects in this group are very unlikely to have any importance for the PPI.

Activities in the other components of the plasma cascade systems were followed indirectly by means of the activity of certain key factors as analyzed by chromogenic substrates (14, 16). Thus, antithrombin III was chosen being the most important inhibitor of the coagulation system. Consumption of AT III would indicate increased activity of this system. Similarly, kallikrein inhibition activity, which mainly reflects C_1 esterase-inhibitor (17), is consumed during activation of complement system (classical pathway; C_1) and activation on the kallikrein-kinin system. This latter system was in addition followed by analyses of kallikrein like activity (KKLA) in plasma. KKLA besides kallikrein also reflects plasmin and factor XII activities (14). Since none of the studied factors showed any changes it can be concluded that the increased pulmonary platelet trapping was

not due to activation of these plasma cascade systems.

The lack of effects on platelet aggregability and on humoral systems favours a central action of the injected β -endorphin. The finding that naloxone, when given centrally, is effective in preventing hypotension in dogs subjected to endotoxin shock in doses that are ineffective when given systemically (18) further supports a central action of β -endorphin. One possible explanation for the PPT inducing effect of centrally administered β -endorphin is a passage of the drug over the blood - brain barrier and hence an increased plasma concentration. This possibility is, however, not very likely. The amount given would, diluted in the intravascular compartment give very low concentrations. Recently, evidence has been put forward that nervous mechanisms might be of importance for pulmonary platelet trapping in shock since denervation of the lung prevented PPT in canine traumatic shock and attenuated canine endotoxin shock (19). It is possible that the centrally administered β -endorphin might induce nervous activity which in turn could induce changes in the pulmonary vascular bed and/or endothelium and consequently platelet trapping. This possibility has not been explored. Another possibility is that centrally infused β -endorphin could stimulate release of endogenous opiates into the systemic circulation from the central nervous system. Still, another possibility is a release of other not yet identified humoral factors which in turn induces platelet pulmonary trapping.

In conclusion, the present study demonstrates that β -endorphin injected into the cerebrospinal fluid in rabbits causes pulmonary platelet trapping. This was not accompanied by changes in the coagulation, fibrinolytic or kallikrein-kinin systems nor in platelet aggregability ex vivo.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (proj. no. 04502), from the Medical Faculty, University of Lund, and from Elsa och Torsten Segerfalks Stiftelse, Helsingborg.

REFERENCES

13

1. Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. and Bloom, F., β -endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 197 (1977) 1367-1369.
2. Rossierr, J., French, E.D., Rivier, C., Ling, N., Guillemin, R. and Bloom, F., Foot-shock induced stress increased β -endorphin levels in blood but not in brain. *Nature* 270 (1977) 618-620.
3. Yamazaki, N., Okabe, E., Hara, A., Takahaski, K. and Ito, H., Pharmacological study on endotoxin shock. 11th Europ Conf Microcirculation. Karger, Basel, 1980, pp 472-476.
4. Holaday, J.W. and Faden, A.J., Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. *Nature* 275 (1978) 450-451.
5. Faden, A.J. and Holaday, J.W., Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. *J Infect Dis* 2 (1980) 229-238.
6. Gurll, N.J., Vargish, T., Reynolds, D.G. and Lechner, R.B., Opiate receptors and endorphins in the pathophysiology of hemorrhagic shock. *Surgery* 89 (1981) 364-369.

7. Albert, S.A., Shires, G.T. III, Illner, H. and Shires, G.T., Effects of naloxone in hemorrhagic shock. Surg Gynecol Obstet 155 (1982) 326-332.
8. Almqvist, P., Kuenzig, M. and Schwartz, S.I., Effect of naloxone on endotoxin-induced pulmonary platelet sequestration. Acta Chir Scand 149 (1983) 23-26.
9. Almqvist, P., Kuenzig, M. and Schwartz, S.I., The effect of naloxone and cyproheptadine on pulmonary platelet trapping, hypotension and platelet aggregability in traumatized dogs. J Trauma 23 (1983) 405-407.
10. Almqvist, P.M., Knigge, K.M., Kuenzig, M., Joseph, S., Pilcher, W. and Schwartz, S.I., Hypotension and pulmonary platelet trapping induced by β -endorphin injection in dogs.
11. Nilsson, I.M., Hemorrhagic and thrombotic disease. John Wiley & Sons, London, 1974.
12. Olow, B. and Nilsson, I.M., Fibrinolysis induced by streptokinase in man. Acta Chir Scand 123 (1962) 247-266.

13. Mussoni, L., Raczka, E., Chmielewska, J., Donati, M.B. and Latallo, Z.S., Plasminogen assay in rabbit, rat and mouse plasma using the chromogenic substrate S-2251. *Thromb Res* 15 (1979) 341-349.
14. Friberger, P., Chromogenic peptide substrates. Their use for the assay of factors in the fibrinolytic and the plasma kallikrein-kinin systems. *Scand J Clin Lab Invest* 42 (1982) Suppl 162.
15. Abrahamsen, A.F., A modification of the technique for ^{51}Cr labelling of blood platelets giving increased circulating platelet activity. *Scand J Haematol* 5 (1968) 53.
16. Aasen, A.O., Kierulf, P. and Strømme, J.H., Methodological considerations on chromogenic peptide substrate assays and application on automated analyzers. *Acta Chir Scand Suppl* 509 (1982) 17-22,
17. Fritz, H., Fink, E. and Truscheit, E., Kallikrein inhibitors. *Fed Proc* 38 (1979) 2753-2759.
18. Janssen, H.F. and Lutherer, L.O., Ventriculocisternal administration of naloxone protects against severe hypotension during endotoxin shock. *Brain Res* 194 (1980) 608-612.

19. Thörne, L.J., Kuenzig, M., McDonald, H.M. and Schwartz, S.I., Effect of denervation of a lung on pulmonary platelet trapping associated with traumatic shock. Surgery 88 (1980) 208-214.

Legend

Systemic arterial blood pressure before and up to 180 min following injection of saline or β -endorphin in the left cerebral ventricle without and with i.v. naloxone.

Fig. 1

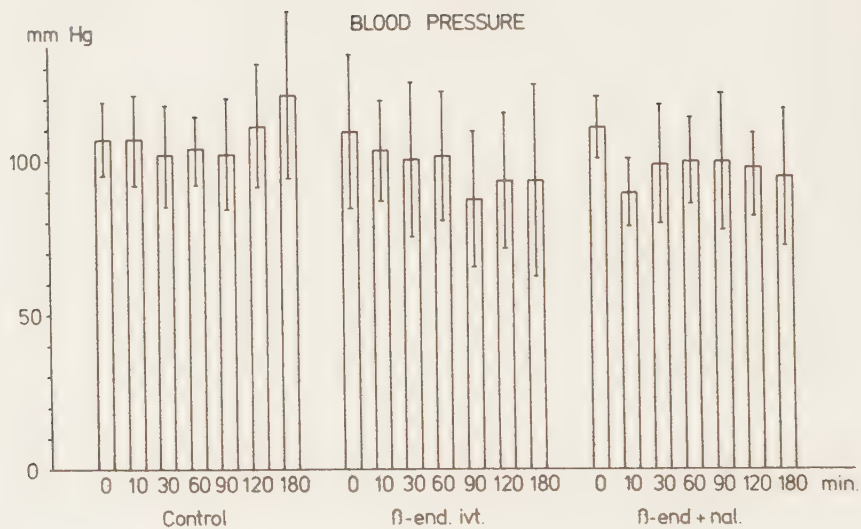


TABLE I. HEART RATE (mean $\pm$ S.D., beats/min)

	<u>n</u>	<u>0</u>	<u>60 min</u>	<u>180 min</u>
Control	5	267 $\pm$ 16	252 $\pm$ 7	270 $\pm$ 26
β -end i.v.t.	6	270 $\pm$ 13	265 $\pm$ 44	265 $\pm$ 12
β -end + nal	5	249 $\pm$ 8	258 $\pm$ 28	264 $\pm$ 13

TABLE II. FIBRINOLYTIC ACTIVITY IN % OF INITIAL VALUE (MEAN $\pm$ SD)

	Control (n=5)	β -endorphin (n=6)	β -endorphin + naloxone (n=5)
10 min	82 $\pm$ 22	71 $\pm$ 28	140 $\pm$ 65
180 min	72 $\pm$ 26	94 $\pm$ 36	13 $\pm$ 18

TABLE III. ANTITHROMBIN III, KALLIKREIN LIKE ACTIVITY AND KALLIKREIN

INHIBITOR AT 180 MIN AFTER β -ENDORPHIN INJECTION.VALUES ARE EXPRESSED AS PER CENT OF BASELINE (mean $\pm$ SD)

Group	n	antithrombin III	kallikrein like activity	kallikrein inhibitor
Control	5	94 $\pm$ 4	95 $\pm$ 6	105 $\pm$ 10
β -endorphin	5	88 $\pm$ 4	107 $\pm$ 18	87 $\pm$ 6
β -endorphin + naloxone	5	95 $\pm$ 7	101 $\pm$ 16	96 $\pm$ 6

TABLE IV. PLATELET AGGREGABILITY IN RESPONSE TO ADP AND COLLAGEN
EXPRESSED AS PER CENT OF INITIAL VALUE (MEAN $\pm$ SD)

<u>ADP</u>			
	Control (n=5)	β -endorphin (n=6)	β -endorphin + naloxone (n=5)
10 min	88 $\pm$ 16	94 $\pm$ 25	96 $\pm$ 16
180 min	95 $\pm$ 22	86 $\pm$ 48	90 $\pm$ 23

<u>COLLAGEN</u>			
	Control (n=5)	β -endorphin (n=6)	β -endorphin + naloxone (n=5)
10 min	105 $\pm$ 44	127 $\pm$ 44	85 $\pm$ 23
180 min	148 $\pm$ 33	130 $\pm$ 85	90 $\pm$ 28

PAPER VI

- TITLE:** Increased Survival of Endotoxin Shocked Dogs Treated with Methylprednisolone, Naloxone and Ibuprofen
- AUTHORS:** Per M. Almqvist, Björn Ekström, Marian Kuenzig, Ulf Haglund and Seymour I. Schwartz
- AFFILIATION:** Departments of Surgery, The University of Rochester, Rochester, New York, Helsingborg Hospital, Helsingborg, Sweden, Malmö General Hospital, Malmö, Sweden.
This study was performed at the University of Rochester, School of Medicine and Dentistry, Strong Memorial Hospital, Rochester, New York.
- CORRESPONDENCE:** Per M. Almqvist, M.D.
Department of Surgery
Strong Memorial Hospital
601 Elmwood Avenue
Rochester, New York 14642
- RUNNING HEAD:** Treatment of canine endotoxin shock

ABSTRACT

The effects of methylprednisolone, naloxone and ibuprofen -alone and in various combinations - on survival, blood pressure, hematocrit, and peripheral platelet and white blood cell counts, were studied in an canine E.coli endotoxin LD₁₀₀ shock model. Treatment was started ten minutes after shock induction. The dogs were kept on a respirator and given intravenous fluids for four hours, then disconnected from the respirator and allowed to recover. Dogs surviving seven days were considered permanent survivors. All control animals died within 36 hours. Five of nine dogs receiving a combined treatment of methylprednisolone, naloxone, and ibuprofen were permanent survivors and showed no macroscopic abnormalities when autopsied. The combined treatment with methylprednisolone and ibuprofen also increased survival. Mortality was delayed in animals treated with methylprednisolone and naloxone. Dogs receiving ibuprofen, a cyclooxygenase inhibitor, had a rapid reversal of hypotension. Hematocrit and platelet and white blood cell counts were similar in all groups.

Key words: shock, endotoxin, methylprednisolone, naloxone, ibuprofen, survival.

INTRODUCTION

Septic shock is a major complication of abdominal surgery, trauma and burns. Although the use of respiratory assistance with positive end expiratory pressure, steroids, broad spectrum antibiotics and carefully titrated fluid resuscitation combined with earlier detection has improved survival, septic shock is still associated with high mortality rates (40 - 70 %) (1,2,3).

Many investigators have assessed the effectiveness of steroid treatment in endotoxin and septic shock. Positive results have been reported by some; others have found steroids ineffective. While some of these differences may be attributed to variations in dosages, in techniques or in the shock model used, steroids alone as adjunct to standard shock treatment do not appear to be sufficient treatment. (4,5)

B-endorphin is now regarded as one of the pathophysiological factors in different types of shock, and naloxone, an opiate antagonist, has been shown to be beneficial in both endotoxin and haemorrhagic shock. (6)

The arachidonic acid derivate, thromboxane A_2 (TxA_2) has recently been indicated as a possible mediator of deleterious effects in shock, (7) and we have shown that ibuprofen, a cyclooxygenase inhibitor, significantly alleviates hypotension and decreases pulmonary platelet trapping (PPT) associated with experimental endotoxin shock. (8)

This study was designed to evaluate the effect of methyl prednisolone, naloxone and ibuprofen in various combinations, on survival in an LD_{100} canine endotoxin shock model.

MATERIAL AND METHODS

Forty-four mongrel dogs of both sexes weighing between 9-14 kg were used. None of the dogs used in this study had signs of obvious respiratory infection, white blood cell count over $17\ 000/\text{mm}^3$ or diarrhea.

Food was withheld the day before the study. The dogs were anesthetized with pentobarbital sodium (30 mg/kg b.w.), endotracheally intubated and connected to a Harvard respirator. The lungs were hyperinflated and the dogs were turned at that time. Normal saline was given intravenously at the approximate rate of 200 ml/hour for five hours. In four or more dogs in each group a catheter was positioned in the femoral and connected to a pressure gauge for continuous blood pressure measurements. A stopcock was inserted in the line for blood sampling. Following baseline blood sampling and recording of control blood pressure, endotoxin shock was induced by an intravenous injection of E.Coli endotoxin (lipopolysaccharide W. E. coli 0128:B12, Difco, Detroit, Michigan) 2 mg/kg b.w. in normal saline over five minutes. Hourly arterial blood samples were taken for hematocrit determination and for peripheral platelet and WBC counts under light microscopy in four or more dogs in each group. Furosemide (Lasix^R) 20 mg was given intravenously two hours postshock induction, to assure that the dogs had diuresis.

The respirator was disconnected at four hours; the dogs were extubated at five hours and returned to the recovery room. No food or water was given on the day following the experiment. On the second day water was allowed, and Nutrical (a high caloric nutritional gel containing protein, EVSCO Pharmaceutical Corp., Buena, N.J.) was given if tolerated and continued until solid food could be taken. All dogs were given up to 500 ml of lactated Ringers solution subcutaneously during the first 24 hours, and this was repeated thereafter until water was tolerated. All animals were turned at least three times a day if they were unable to stand. Survivors were sacrificed on the seventh day; heart, lungs, kidneys and the intra-abdominal organs were examined macroscopically.

The following groups were studied: Ia, endotoxin shock controls; Ib, shock controls without furosemide, hyperinflation of the lungs, or turning, and with minimal intravenous saline during the five hours (survival study only); II, shock plus methylprednisolone alone (MP); III, shock plus MP and naloxone hydrochloride (N); IV, shock plus MP sodium ibuprofen (I); V, shock plus N and I; VI, shock plus MP, N, and I.

All drugs were injected intravenously. MP (Solu-Medrol^R, Upjohn Co., Kalamazoo, Mich.) 30 mg/kg b.w. was given 10 minutes and five

hours post shock induction. N (Endo Laboratories, Garden City, N.Y.) 2 mg/kg b.w. was given 10 minutes post shock and repeated hourly for five hours. I (Motrin^R, Upjohn, Crawley, Sussex, England) 5 mg/kg b.w. was given 10 minutes postshock.

Statistical analysis was done with the Mann-Whitney U-test for unpaired data for comparison between the treated groups and group Ia. A p-value of less than 0.05 was considered significant.

RESULTS

Blood pressure: The initial blood pressure drop recorded at 10 minutes postendotoxin injection was similar in all groups with no significant difference. Group Ia, endotoxin control, remained low throughout the five hour period. Group II had a lesser degree of blood pressure drop at five hours, but this was not significantly different from group Ia. Group III had a significantly higher blood pressure at five hours compared to group Ia. All the ibuprofen treated groups IV, V and VI had a fast recovery of blood pressure; it was already significantly higher than group Ia at 30 minutes postshock (Fig. 1).

Hematocrit (Hct): The Hct rose in all groups and the rise recorded at five hours is not significantly different in the treated groups compared to endotoxin controls (Table I).

Peripheral Platelet Counts: All groups had a similar drop in circulating platelets by one hour. The per cent drop from baseline value recorded at five hours was significantly greater in Group IV compared to endotoxin control. The other treated groups did not differ significantly from endotoxin control (Table I).

Peripheral WBC Counts: The initial drop in circulating leucocytes recorded at one hour was similar in all groups. The percent drop from baseline values recorded at five hours was significantly less in Group VI compared to that of Group Ia (Table I).

Diarrhea: The incidence of diarrhea, especially bloody diarrhea, was less in all treated groups compared to endotoxin controls.

Survival Time: In the group Ia two of five dogs survived more than 24 hours but all died before 36 hours (Table II). In group Ib, which did not receive fluid or Furosemide and was not turned or sighed, three dogs died before eight hours and all were dead by 16 hours. Group II (MP only) had survival time similar to group Ia. In group III (MP + N) four of five dogs survived more than 24 hours and there was one permanent survivor. In group IV (I + MP) six of nine survived more than 24 hours and there were three permanent survivors. (One dog died on the sixth day due to treatment of an extraneous condition and is regarded as a permanent survivor). In group V (N + I) one dog of seven survived more than 24 hours but none lived 48 hours. In group VI (N + I + MP) only two were dead within 24 hours and there were five permanent survivors from a total of nine.

Autopsy: There were no macroscopic abnormalities seen in any of the animals surviving to seven days.

DISCUSSION

The results of this survival study shows that the combined treatment with naloxone, ibuprofen and methylprednisolone is effective in preventing death in this otherwise lethal E.coli endotoxin shock model. There were five permanent survivors of nine dogs treated with drug combination. These dogs appeared normal, they were eating, had no diarrhea and showed no macroscopic pathological signs in the lungs, kidneys, intestine, or liver when autopsied after seven days. Untreated endotoxin shocked dogs all died within sixteen hours. I.v. infusion of fluids, diuretics, periodic hyperinflation of the lungs and turning the animal did not prolong survival beyond thirty hours.

The proposed effects of steroids in experimental shock include a positive inotropic action (9), protection against platelet and leucocyte aggregation (10), and stabilization of lysosomal membranes (11). Steroids have been shown to lessen the increase in lung vascular permeability in endotoxin shocked sheep (11). Steroids, however, are also reported to have a negative effect on the leucocytes' ability to phagocytize. This might be an explanation for some of the negative results regarding the effects of steroids in various shock situations, since this latter effect may predominate if the steroids are instituted late in the shock state (12).

There is still considerable controversy about the efficacy of steroid treatment in clinical shock and sepsis. Absence of improvement in steroid treated patients is discussed by Blaisdell who stresses the disadvantages of steroids, particularly the short time interval in which they might be helpful, and also the deleterious results which may follow extended treatment or treatment given at an inappropriate time (13). Schumer's double-blind study of 172 septic shock patients divided randomly into either MP or saline control groups presents a 10 % mortality for the MP group and 38 % for non-steroid group (14). In a more recent report he suggests that the anti-complement properties of certain steroids may be a positive factor in the treatment of endotoxemia (15). A discussion of these two opposing viewpoints and the differences seen by their proponents is given by Hess and Manson. They assign the positive aspects of steroid treatment to inhibition of leukocyte mediation or cardiovascular problems, by the mechanism of oxygen radical inhibition. The narrow window for steroid treatment initiation and the potential for opportunistic secondary infections is also emphasized (12).

In our experimental model methylprednisolone alone did not prolong survival time. Only regimens including MP, however, resulted in permanent survivors.

Increased circulating levels of B-endorphin have been shown in rats subjected to trauma and to endotoxin shock (16, 17). B-endorphin injected into an isolated stomach segment resulted in cell injury similar to that caused by endotoxin injection (18). Centrally administered B-endorphin causes hypotension and bradycardia (19, 20) and we have also shown that it caused PPT (20). Naloxone, an opiate antagonist, has proved helpful in preventing both the hypoglycemia and acidosis associated with endotoxin shock in dogs, and in reducing PPT and hypotension in dogs subjected to trauma or to endotoxin shock (21, 22, 23). In our study when naloxone was added to the MP treatment, mortality was delayed.

Methylprednisolone, in addition to previously discussed effects, also has a negative feedback effect on the release of both ACTH and B-endorphin, which could prevent further release of B-endorphin but would not affect that already in circulation. The additive effect of naloxone could be explained by blockade of this circulating B-endorphin at the receptor level.

Ibuprofen, a cyclooxygenase inhibitor, blocks the formation of arachidonic acid derivatives synthesized via this pathway, (prostaglandin and thromboxane (TxA₂). Thromboxane increases platelet aggregation and, in high doses, also aggregation of leukocytes while prostacyclin has been reported to have the opposite effect (10). We have recently shown ibuprofen to be effective in reducing both hypotension and PPT in endotoxin shocked dogs (8). In addition, ibuprofen has proved effective in improving cardiac performance and in reducing acidosis in dogs subjected to endotoxin shock (24).

Treatment with MP + I increased survival time and all groups given ibuprofen had rapid reversal of hypotension. Hammerschmidt has recently shown that MP and I have a synergistic effect in reducing the ability of complement activated leukocytes to aggregate and to release oxygen radicals, which may be one of the major factors in mediating endothelial cell damage in the lungs (25).

Activation of complement and trapping of platelets and leukocytes seem to play an important role in the complex etiology of the lung injury (10). The release of vasoconstrictors, such as serotonin and TxA₂ from platelets contributes to further aggregation (26, 27, 28). Complement activated leukocytes have been shown to destroy endothelial cells *in vitro*, probably by releasing oxygen free radicals, and this capability is significantly augmented when platelets are present (10).

Although all the treated groups had a greater return of leukocytes to the peripheral circulation at five hours than the endotoxin controls, this return does not correlate with survival, nor does the return of platelets at five hours. This supports the view that the degree of mechanical trapping of these blood elements is not the single prime factor in the injuries sustained in shock, and stresses the importance of other deleterious agents from these cells, such as TxA₂ and oxygen radicals.

Bloody diarrhoea is a common feature in dogs receiving endotoxin; the incidence of bloody diarrhea in all the treated groups was less than in the endotoxin controls. This may be an indication that these dogs have an improved microcirculation in the intestine, probably due to a higher blood pressure. The pathogenesis of the intestinal mucosal damage has been thoroughly studied in different shock states in cats, and has been shown to be correlated to the degree of

hypotension, leading to hypoxia of the villus tip due to increased shunting of oxygen in the intestinal counter current exchange mechanism (29, 30). Evidence have also been presented that cardio-toxic material is released from ischemic intestine, which leads to impaired cardiac function and further hypotension (28).

In survival studies using a model comparable to ours treatment with methylprednisolone increased survival time but no dogs lived beyond 24 hours (4). High doses of naloxone improved survival time when used in a similar model, and two of eleven dogs were regarded as permanent survivors at 96 hours (21). To our knowledge no survival studies have yet been published using ibuprofen.

We feel that the results of this study are promising and we believe that this treatment combination merits further trials on other experimental models to test the validity of this therapy as adjunct to traditional shock therapy.

ACKNOWLEDGMENTS

This study was supported by N.I.H. Grant No. HL 13466.

Methylprednisolone and ibuprofen were kindly supplied by the Upjohn Company, Kalamazoo, Michigan. Naloxone was kindly supplied by the Endo Laboratories, Garden City, N.Y.

The authors are grateful to Roger M. Bender, Barbara Folsom, and Robert Baitchman for technical assistance, and for help in postoperative care of the animals.

REFERENCE LIST

1. Wosornu L: Septic shock. *Trop Doc* 9:61-66, 1979.
2. Landesman SH, Gorbach SL: Gram negative sepsis and shock. *Orthop Clin North Am* 9:611-625, 1978.
3. Clowes GHA, Hirsch E, Williams L, Kwasnik E, O'Donnell TF, Cuvas P, Saini VK, Moradi J, Farizan M, Saravis C, Stone M, Kuffler J: Septic lung and shock lung in man. *Ann Surg* 181:681-692, 1975.
4. White GL, Archer LT, Beller BK, Hinshaw LB: Increased survival with methylprednisolone treatment in canine endotoxin shock. *J Surg Res* 25:357-364, 1978.
5. Hinshaw LB, Coalson JJ, Benjamin BA, Archer LT, Keller BK, Kling OR, Hasser EM, Phillips RW: Escherichia coli shock in the Baboon and the response to adrenocorticosteroid treatment. *Surg Gynecol Obstet* 147:545-557, 1978.
6. Faden AJ, Holoday JW: Experimental endotoxin shock: The pathophysiologic function of endorphins and treatment with opiate antagonists. *J Infect Dis* 142:229-238, 1980.
7. Cook JA, Wise WC, Halushka PV: Elevated thromboxane levels in the rat during endotoxin shock. *J Clin Invest* 65:227-230, 1980.
8. Almqvist PM, Kuenzig M, Schwartz SJ: Treatment of canine endotoxin shock with ibuprofen, a cyclooxygenase inhibitor. Submitted for publication.
9. Bowen JM: Ane corticosteroids useful in shock therapy. *JAVMA* 177:453-455, 1980.
10. Jacob HS, Moldow CF, Flynn PJ, Weisdorf DJ, Vercellotti GM, Hammerschmidt DE: Therapeutic ramifications of the interaction of complement granulocytes and

platelets in the production of acute lung injury. *Ann NY Acad Sci* **384**:489-495, 1982.

11. Demling RH, Proctor R, Grossman J, Duy N, Starling J: Comparison of the systemic and pulmonary vascular response to endotoxin with plasma and lung lymph lysosomal enzyme release: Effect of steroid pretreatment. *Circ Shock* **7**:317-331, 1980.
12. Hess ML: The paradox of steroid therapy: Inhibition of oxygen free radicals. *Circ Shock* **10**:1-5, 1983.
13. Blaisdell FW: Controversy in shock research. Con: the role of steroids in septic shock. *Circ Shock* **8**:673-682, 1981.
14. Schumer W: Steroids in the treatment of clinical septic shock. *Ann Surg* **184**:333-341, 1976.
15. Schumer W: Controversy in shock research. Pro: the role of steroids in septic shock. *Circ Shock* **8**:667-761, 1981.
16. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F: B-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* **136**:1367-1369, 1977.
17. Yamazaki N, Okabe E, Hara A, Takahashi K, Ito H: Pharmacological study on endotoxin shock. 11th Europ Conf Microcirculation, Garmisch-Partenkirchen 1980. *Bibliotheca anat* No 20:472-476, 1981.
18. Rees M, Payne JG, Bowen JC: Naloxone reverses tissue effects of live *Escherichia coli* sepsis. *Surgery* **91**:81-86, 1982.
19. Laubie M, Schmitt H, Vincent M, Remond G: Central cardiovascular effects of morphinomimetic peptides in dogs. *Eur J Pharmacol* **46**:67-71, 1977.
20. Almqvist PM, Kuenzig M, Knigge KM, Schwartz SJ: Association of centrally administered B-endorphin and adult respiratory distress syndrome. Manuscript.

21. Raymond RM, Harkema JM, Stoffs WV, Emerson TE: Effects of naloxone therapy on hemodynamics and metabolism following a superlethal dosage of *Escherichia coli* endotoxin in dogs. *Surg Gynecol Obstet* **152**:159-162, 1981.
22. Almqvist PM, Kuenzig M, Schwartz SJ: The effect of naloxone and cyproheptadine on pulmonary platelet trapping, hypotension and platelet aggregability in traumatized dogs. Accepted in *Journal Trauma*.
23. Almqvist PM, Kuenzig M, Schwartz SI: Effect of naloxone on endotoxin-induced pulmonary platelet sequestration. *Acta Chir Scand* **149**:23-26, 1983.
24. Jacobs ER, Soulsby ME, Bone RC, Wilson FJ, Jr, Hiller FC: Ibuprofen in canine endotoxin shock. *J Clin Invest* **70**:536-541, 1982.
25. Hammerschmidt DE, Flynn PJ, Coppo PA, Skubitz KM, Jacobs HS. Synergy among agents inhibiting granulocyte aggregation. *Inflammation* **6**:169-176, 1982.
26. Ljungqvist U, Schwartz SI: Pulmonary platelet trapping during shock and pulmonary embolism. *J Surg Res* **18**:559-565, 1975.
27. Myrvold HE, Brandberg A, Lindqvist O, Busch C, Lewis DH: The role of platelets and fibrinogen in experimental septic shock. *Acta Chir Scand* **143**:131-137, 1977.
28. Best LC, Holland TK, Jones PBB, Russell RGG: The interrelationship between thromboxane biosyntheses, aggregation and 5-hydroxytryptamine secretion in human platelets in vitro. *Thromb Haemost* **43**:38-40, 1980.
29. Lundgren O, Haglund U: The pathophysiology of the intestinal countercurrent exchanger. *Life Sci* **23**:1411-1422, 1978.
30. Haglund U, Falk A, Haglund E, Myrvold HE, Lundgren O: An evaluation of the functional implications of the

intestinal mucosal lesions in shock. Adv Shock Res **8**:91-97, 1982.

31. Haglund U, Lundgren O: Intestinal ischemia and shock factors. Fed Proc **37**:2729-2733, 1978.

TABLE I

Hematocrit, Platelet, and WBC Results^o

Group	n	Hematocrit (Increase at 5 hours)	Platelet (% drop at 5 hours)	WBC (% drop at 5 hours)
Ia	5	13 $\pm$ 2	25 $\pm$ 16	72 $\pm$ 7
II	5	14 $\pm$ 10	47 $\pm$ 15	57 $\pm$ 27
III	4	10 $\pm$ 3	34 $\pm$ 16	51 $\pm$ 12
IV	5	11 $\pm$ 6*	50 $\pm$ 14	54 $\pm$ 29
V	4	13 $\pm$ 9	41 $\pm$ 33**	32 $\pm$ 17
VI	5	9 $\pm$ 5	15 $\pm$ 20	33 $\pm$ 31

^o Mean $\pm$ SD

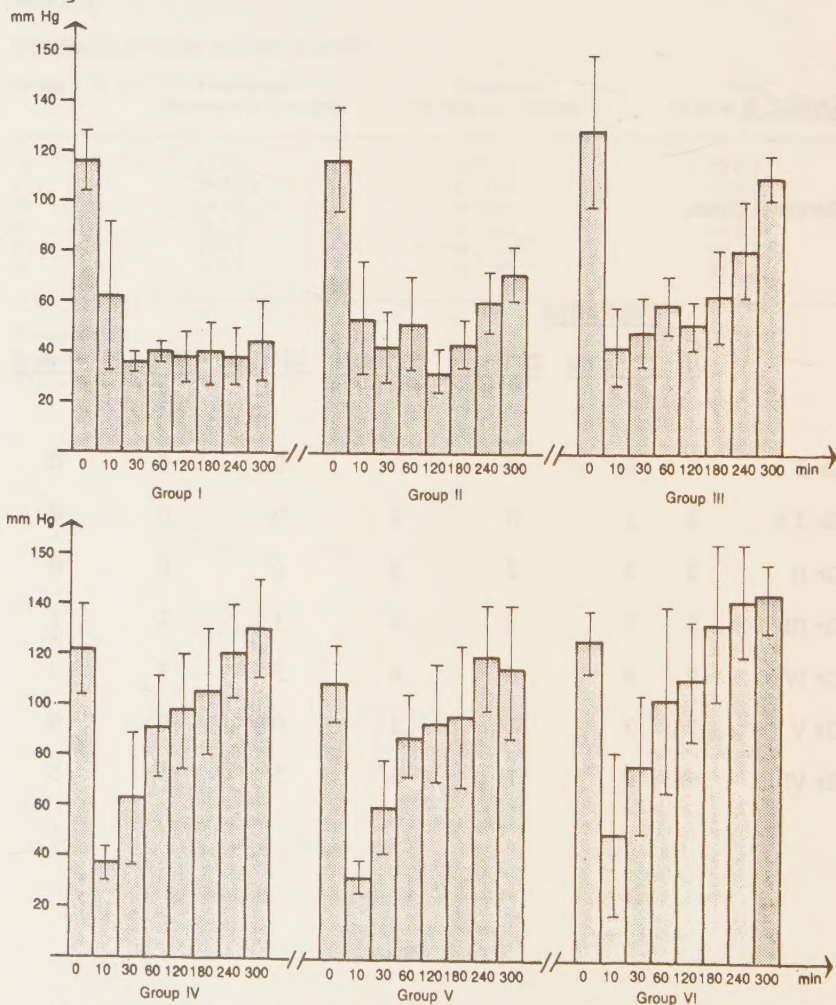
* n = 6

** n = 5

TABLE II**Survival time.**

	<u>n</u>	<u>Dogs alive</u>					
		<u>12 hours</u>	<u>24 hours</u>	<u>36 hours</u>	<u>48 hours</u>	<u>72 hours</u>	<u>7 days</u>
Gr 1 a	5	4	2	0	0	0	0
Gr 1 b	4	1	0	0	0	0	0
Gr II	5	5	2	0	0	0	0
Gr III	5	5	5	4	1	1	1
Gr IV	9	8	6	6	5	3	3
Gr V	7	7	1	1	0	0	0
Gr VI	9	9	7	5	5	5	5

Fig. 1



Mean blood pressure ($\pm$ S.D.) before and after shock induction. Group III is significantly higher than control at five hours. Groups IV, V and VI are significantly higher than the control by 30 minutes (see text for details).

